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(54) Title: USE OF SURFACTANTS FOR INTRODUCING GENETIC MATERIAL INTO LUNG CELLS

(57) Abstract

A process for introducing genetic material (wich may be contained in an expression vehicle such as an adenoviral vector or retroviral vector) into lung cells which comprises contacting the lung cells with the genetic material and at least one surfactant. The at least one surfactant may be a lipid-containing surfactant. Such process provides for improved transduction of the genetic material into lung cells, as well as for transduction of both large airway (trachea and bronchus) cells and lung parenchymal cells.

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USE OF SURFACTANTS FOR INTRODUCING GENETIC MATERIAL INTO LUNG CELLS

This application is a continuation-in-part of application Serial No. 08/414,488, filed March 31, 1995.

This invention relates to the introduction of genetic material into cells, and in particular into lung cells. More particularly, this invention relates to improving the introduction of genetic material into cells, such as, for example, lung cells, (either *in vivo* or *ex vivo* or *in vitro*) by contacting the lung cells into which the genetic material is to be introduced with the genetic material and a surfactant.

BACKGROUND OF THE INVENTION

The development of strategies for the transfer of genes into desired cells offers new therapeutic possibilities.

Gene delivery vehicles which may be employed include retroviral vectors and adenoviral vectors. Retroviral vectors may be employed for infecting dividing cells, while adenoviral vectors may be employed for infecting dividing and non-dividing cells. Adenoviral vectors have been used successfully for in vivo gene transfer of marker genes such as ß-galactosidase (Stratford-Perricaudet, *et al.*, Hum. Gene Ther. Vol. 1, pgs. 240-256 (1990); Mastrangeli, *et al.*, J. Clin. Invest., Vol. 91, pgs. 225-234 (1993);

Englehart, *et al.*, Nat. Genet., Vol. 4, pgs. 27-34 (1993); Yei, *et al.* Hum. Gene Ther., Vol. 5, pgs. 733-746 (1994)), the luciferase gene (Trapnell, Adv. Drug. Del. Rev., Vol. 12, pgs. 185-199 (1994)), as well as potentially therapeutic genes such as ornithine transcarbamylase (Stratford-Perricaudet, *et al.*, 1990), α-1-antitrypsin (Rosenfeld, *et al.*, Science, Vol. 252, pgs. 431-434 (1991)), cystic fibrosis transmembrane conductance regulator, (Rosenfeld, *et al.*, Cell, Vol. 68, pgs. 143-155 (1992); Dabner, *et al.*, Nature Genet., Vol. 1, pgs. 75-83 (1993)); Factor IX (Smith, *et al.*, Nature Genet., Vol. 5, pgs. 397-402 (1993)), dystrophin (Vincent, *et al.*, Nat. Genet., Vol. 5, pgs. 130-134 (1993)) and chimeric inhibitors of tumor necrosis factor (Kolls, *et al.*, Proc. Nat. Acad. Sci., Vol. 91, pgs. 215-219 (1994)), to a variety of organs and tissues.

One therapeutic possibility for the transfer of genes into desired cells is the treatment of cystic fibrosis.

The basic defect of cystic fibrosis is an abnormality of cAMP regulated chloride secretion by epithelial cells, affecting exocrine glands and the lungs. The disease is characterized by abnormal secretions in respiratory, gastrointestinal, and reproductive tracts and in sweat glands. The failure of cAMP stimulated chloride secretion by epithelial cells leads to dehydration of secretions which become viscid and concentrated. Cystic fibrosis is inherited as an autosomal recessive disease, and the CFTR gene is located on the long arm of Chromosome 7 at position 7q31 (Rommens, *et al.*, Science, Vol. 245, pgs. 1059-1065 (1989); Riordan, *et al.*, Science, Vol. 285, pgs. 106-109 (1989); Kerem, *et al.*, Science, Vol.245, pgs. 1073-1080 (1989). More than 200 mutations have been recognized at this locus. Although the disease process is a generalized disorder of exocrine secretion, the lung disease accounts

for most of the morbidity and mortality (Wood *et al.*, Am. Rev. Respir. Dis., Vol. 113, pgs. 833-878 (1976)).

The human CFTR is a 1480 amino acid polypeptide (Mr=168,021) with structural domains characterized by distinct hydrophobic regions each corresponding to transmembrane segments (Kerem, *et al.*, 1989). A putative regulatory, or R domain, and two nucleotide binding folds in which resides the Δ508 mutation (fold 1) are characteristic of the CFTR polypeptide in all species studied to date. The CFTR protein is membrane bound, binds to and hydrolyses ATP, and is both a phosphoprotein and a glycoprotein (Kerem, *et al.*, 1989, Riordan, *et al.*, 1989, Cheng, *et al.*, Cell, Vol. 66, pgs. 1027-1036 (1991), Gregory, *et al.*, Nature, Vol. 341, pgs. 382-386 (1990)). Transfer of the full-length cDNA encoding CFTR confers cAMP-dependent chloride pump activity that will complement the chloride transport defect characteristic of cells derived from cystic fibrosis patients (Rich, *et al.*, Nature, Vol. 347, pgs. 358-363 (1990), Drumm, *et al.*, Cell, Vol. 62, pgs. 1227-1233 (1990)). Evidence to date strongly supports the role of the CFTR as a chloride transporting protein. The hCFTR cDNA has been transferred to a variety of cystic fibrosis and non-cystic fibrosis cell types wherein the CFTR protein was identified immunohistochemically and associated with the generation of cAMP-dependent chloride transport activity (Whitsett, *et al.*, Nature Genetics, Vol. 2, pgs. 13-20 (1992)).

In patients with cystic fibrosis, lung development proceeds normally until after birth, when mucous plugging, air trapping and infection begin to cause chronic lung injury that usually accelerates in the second decade of life (Wood, *et al.*, 1976). Primary therapy may not be considered by transferring the wild-type hCFTR cDNA to the epithelial cells of the respiratory tracts of patients with cystic fibrosis. Biochemical evidence to date confirms the

clinical observation that cystic fibrosis is a recessive trait and that expression of the mutant CFTR does not confer a dominant abnormality of cellular function in cells transfected with CFTR. Thus, it is likely that restoration of cAMP-dependent chloride transport, or other more subtle abnormalities of cellular function such as abnormal acidification of intracellular organelles (Barasch, *et al.*, Nature, Vol. 352, pgs. 70-73 (1991)), may be accomplished by transfer of the CFTR gene to cells of the lung. The CFTR gene is expressed at very low levels in the respiratory tract in both normal individuals and in individuals with cystic fibrosis (Trapnell, *et al.*, Proc. Nat. Acad. Sci., Vol. 88, pgs. 6565-6569 (1991), Tresize, *et al.*, Nature, Vol. 353, pgs. 434-437 (1991)) and pulmonary epithelial cells express human CFTR at levels below that readily detected by *in situ* hybridization or immunocytochemistry. The small airways are involved early in the pathogenesis of the disease resulting in the hyperaeration on the chest radiograph commonly seen within the first year of life. The clinical findings in cystic fibrosis, therefore, focus attention to the need to complement the CFTR in the epithelial cell lining of the bronchial and bronchiolar regions of the lung at an early age, before mucous plugging and infection cause irreversible lung disease and make access via the respiratory tract difficult. Recent work by Johnson *et al.*, Nature Genetics, Vol. 2, pgs. 21-25 (1992) demonstrated that ion conductance across CF epithelial cell monolayers could be corrected by expression of CFTR mRNA in only 6-10% of the cells comprising the monolayer. The CFTR mRNA is present in virtually all epithelial cell types of the respiratory tract, providing a broad target for expression of the transferred CFTR gene.

Strategies which may be applicable for gene transfer to the respiratory epithelium *in vivo* include the use of

viral vectors including retroviruses, adenoviruses, adeno-associated viruses, plasmids, liposomes or DNA-carrier complexes. Because adenovirus is efficient and able to infect a wide variety of pulmonary epithelial cells *in vivo* and *in vitro*, this type of vector appears suitable for transfer of the CFTR gene to the respiratory tract (Rosenfeld, *et al.*, Cell, Vol. 68, pgs. 143-155 (1992)). Receptor systems endogenous to the respiratory tract or viral proteins which might aid in the delivery and integration of the DNA into the respiratory tract are also being developed (Curiel, *et al.*, J. Respir. Cell. Mol. Biol., Vol 6, pgs. 247-252, (1992)).

The respiratory epithelium of the adult lung will express gene products transiently following exposure to vectors given by either direct tracheal instillation (Mastrangeli, *et al.*, J. Clin. Invest., Vol. 91, pgs. 225-255, (1993)) or by aerosol (Stribling, *et al.*, Proc. Nat. Acad. Sci., Vol. 89, pgs. 11227-11281, (1992)). The amount of expression in larger and more proximal airways was vector dose dependent when a small volume of fluid was used for viral administration to cotton rats.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention now will be described with respect to the drawings, wherein:

Figure 1 is a schematic of the construction of plasmid pHR;

Figure 2 is a schematic of the construction of an expression vehicle including an adenoviral ITR, an encapsidation signal, a Rous Sarcoma Virus promoter, an adenoviral tripartite leader sequence, and linking sequences;

Figure 3 is a schematic of the construction of plasmid pAvS6;

Figure 4 is a map of plasmid pAvS6;

Figure 5 is a map of plasmid pGEM-luc;

Figure 6 is a map of plasmid pAvS6-luc;

Figure 7 is a schematic of adenoviral vector Av1Luc1;

Figure 8 is a graph of expression of luciferase in the total lung tissue of rabbits at three days after tracheal instillation of adenovirus including the luciferase gene;

Figure 9 is a graph of expression of luciferase in the lung parenchyma of rabbits at three days after tracheal instillation of adenovirus including the luciferase gene;

Figure 10 is a graph of expression of luciferase in the trachea and bronchus of rabbits at three days after tracheal instillation of adenovirus including the luciferase gene;

Figure 11 is a graph of the percentage of total luciferase expression in the trachea and bronchus of rabbits at three days after tracheal instillation of adenovirus including the luciferase gene;

Figure 12 is a graph of the effect of surfactant concentration on luciferase expression in rabbits at three days after tracheal instillation of adenovirus including the luciferase gene;

Figure 13 is a graph of total lung expression of luciferase in rabbits at 3 days, 7 days, and 14 days after intratracheal instillation of adenovirus inducing the luciferase gene;

Figures 14A and 14B are graphs of radioactivity per volume in reservoir of aerosolizer relative to that measured before aerosolization. In Figure 14A, the aerosolizer was loaded with 10 ml of saline that contained ^{99}Tc-sulfur colloid and the ^{33}P-labeled adenovirus Av1Luc1 vector. Aerosolization occurred for the times indicated on the x-axis. Aerosolization was interrupted for sampling of the residual volume in the reservoir. After 120 min. approximately 5 ml had been aerosolized. In Figure 14B, the measurements were repeated with 10 ml of saline

containing ^{3}H-surfactant at 10 mg/ml in addition to the other radiolabeled materials;

Figures 15A, 15B, and 15C are graphs of recovery of aerosols on plates of a cascade impactor. Aerosols of saline containing the indicated radiolabeled materials in Figure 15A and Figure 15B were collected for 5 min. intervals spaced by 15 min. of aerosolization such that the 4 collections occurred during 120 min. of total aerosolization time. The percent recoveries of the radiolabels on the plates with the indicated effective cut-off diameters and from the cyclone (effective cut off diameter <6.3 microns) for the four collections were averaged. There were no differences in percent recoveries between the radiolabels for the aerosols. Surfactant did not change the size distribution of the particles as shown in Figure 15C;

Figures 16A and 16B are graphs of distributions of ^{99m}Tc-sulfur colloid immediately after instillation and aerosolization to groups of 5 rabbits for each treatment. As shown in Figure 16A, aerosolization without surfactant resulted in less relative recovery of ^{99m}Tc-sulfur colloid in parenchyma and more in airways than did instillation. As shown in Figure 16B, the same pattern of distributions occurred with aerosolization or instillation of suspensions containing 10 mg/kg surfactant;

Figures 17A and 17B are graphs of lobar distributions of ^{99m}Tc-sulfur colloid immediately after instillation and aerosolization to groups of 5 rabbits for each treatment. There were no differences in distribution to lung lobes resulting from instillation relative to aerosolization (Figures 17A and 17B) and surfactant did not alter lobar distributions; and

Figures 18A and 18B are graphs of luciferase activity 3 days after treatment of rabbits with aerosols or by instillation to groups of 5 rabbits for each treatment.

Surfactant did not alter the percent distribution of transgene expression (Figures 18A and 18B). Instillation of the vector resulted in more relative transgene expression in parenchyma and less in the airways than did aerosolized vector.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with an aspect of the present invention, there is provided a process for introducing genetic material into lung cells. The process comprises contacting the lung cells with the genetic material and at least one surfactant.

Surfactants which may be employed include those which are compatible with the tissues and cells of the respiratory system, including the trachea, bronchus, and lung, including the alveoli and lung parenchymal cells.

In one embodiment, the at least one surfactant is a lipid-containing surfactant. The term "lipid-containing surfactant" as used herein means that the surfactant includes at least one lipid. Such lipid-containing surfactants may contain one or more of the following materials which include long chain carboxylic acids; long chain carboxylic acid esters; long chain carboxylic acid alcohols; phospholipids; glycolipids; and neutral lipids.

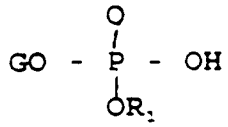
The long chain carboxylic acids, generally contain at least 4 carbon atoms and preferably contains at least 12 carbon atoms, most preferably 14 to 18 carbon atoms. Such long chain carboxylic acids thus include the fatty acids, including extended chains of fatty acids. In some cases this carbon chain is fully saturated and unbranched, while others contain one or more double bonds. A few contain 3-carbon rings or hydroxyl groups. Examples of saturated straight chain acids are n-dodecanoic acid, n-tetradecanoic acid, n-hexadecanoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid. Also useful are unsaturated monoolefinic straight

chain monocarboxylic acids. Examples of these are oleic acid, gadoleic acid and erucic acid. Also useful are unsaturated (polyolefinic) straight chain monocaboxyic acids. Examples of these are linoleic acid and linolenic acid.

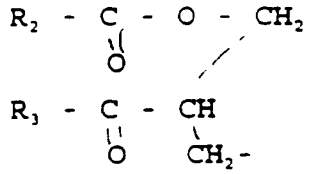
In general, the long chain carboxylic acid esters include at least 4 carbon atoms, and preferably include at least 12 carbon atoms, and more preferably include from about 14 to about 18 carbon atoms. Such long chain carboxylic acid esters include, but are not limited to, monoglycerides, diglycerides and triglycerides, such as glyceryl monostearates; glyceryl monopalmitates; mixtures of glyceryl monostearate and glyceryl monopalmitate (Myvaplex 600, Eastman Fine Chemical Company); glyceryl monolinoleate; glyceryl monooleate; mixtures of glyceryl monopalmitate, glyceryl monostearate glyceryl monooleate and glyceryl monolinoleate (Myverol 18-92, Eastman Fine Chemical Company); glyceryl monolinolenate; glyceryl monogadoleate; mixtures of glyceryl monopalmitate, glyceryl monostearate, glyceryl monooleate, glyceryl monolinoleate, glyceryl monolinolenate and glyceryl monogadoleate (Myverol 18-99, Eastman Fine Chemical Company); acetylated glycerides such as distilled acetylated monoglycerides (Myvacet 5-07, 7-07 and 9-45, Eastman Fine Chemical Company); mixtures of mono- and di-glyceride esters such as Atmul (Humko Chemical Division of Witco Chemical); ethoxylated mono- and di-glycerides; lactated mono- and di-glycerides; lactylic esters of long chain carboxylic acids; and polyglycerol esters of long chain carboyxlic acids.

Long chain carboxylic acid alcohols which may be employed include, but are not limited to, the hydroxyl forms of the long chain carboxylic acids hereinabove described.

In general, phospholipids which may be employed may have the following structure:



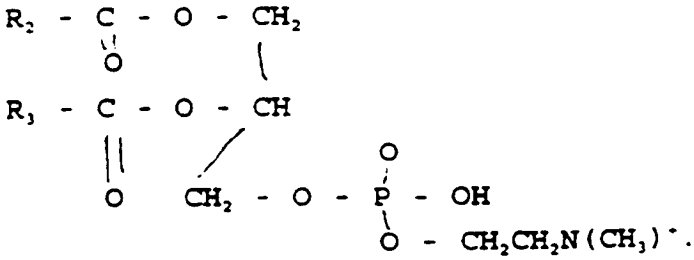
Wherein G is



Each of R_2 and R_3 is an aliphatic hydrocarbon having from 4 to 18 carbon atoms, preferably from 14 to 18 carbon atoms, and R_2 and R_3 may be the same or different.

R_1 is selected from the group consisting of an alkyl group having from 2 to 18 carbon atoms; an alcohol including polyhydroxy alcohols and preferably an alkanol or alkane diol or triol having from 2 to 18 carbon atoms; an alkylamine having from 2 to 18 carbon atoms; choline, which has the structure $CH_2CH_2N(CH_3)_3^+$; and an amino acid.

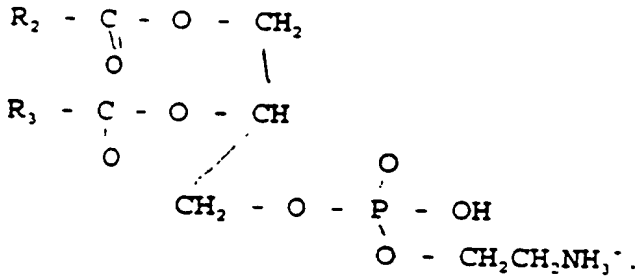
In one embodiment, R_1 is choline. Thus, the phospholipid is a phosphatidylcholine, which has the following structure:



Because the - OH moiety on the phosphorus is highly acidic, the ester exists mostly in the ionic form as a dipolar ion.

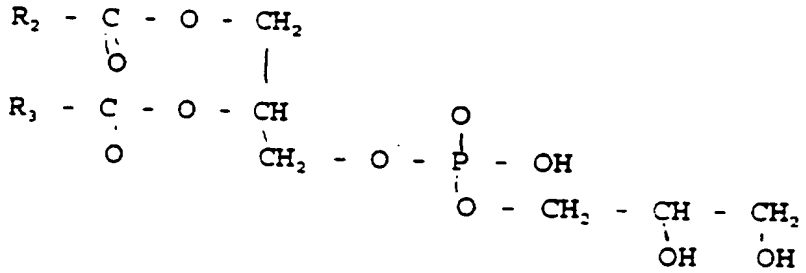
In another embodiment, R_1 is an alkylamine, preferably ethylamine, and the resulting phospholipid is a

phosphatidyl ethanolamine, which has the following structure:



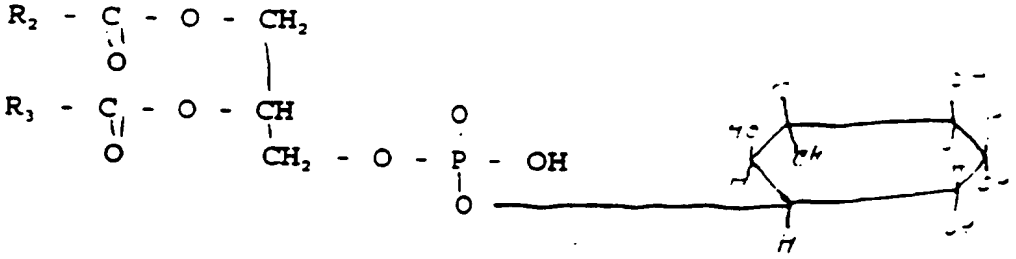
As hereinabove mentioned, the ester may exist in ionic form as a dipolar ion.

In another embodiment, R_1 is an alcohol and in particular a polyhydric alcohol. In one embodiment, the phospholipid is phosphatidylglycerol, which has the following structure:



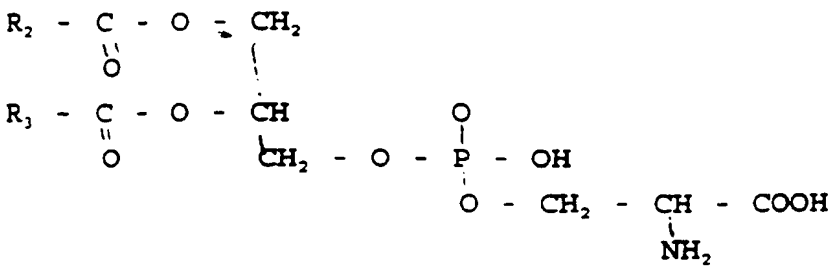
The ester may exist in ionic form as a dipolar ion.

In another embodiment, the phospholipid is phosphatidylinositol, which has the following structure:



The ester may exist in ionic form as a dipolar ion.

In another embodiment, R_1 is an amino acid. In one embodiment, the phospholipid is phosphatidylserine, which has the following structure:



The ester, as well as the amine and carboxyl groups of the amino acid, may exist in ionic form.

Neutral lipids include, but are not limited to, triglycerides, and sterols and sterol esters, such as, for example, cholesterol and cholesterol esters, cholestanol and cholestanol esters, and lanosterol and lanosterol esters.

In one embodiment, the lipid-containing surfactant includes at least one phospholipid, at least one neutral lipid, at least one fatty acid, and at least one lung surfactant protein selected from the group consisting of surfactant protein B (SP-B) and surfactant protein C (SP-C). Preferably, such surfactant further includes palmitylphosphatidyl choline, and may even further include palmitic acid, and glycerol tripalmitate. More preferably, such surfactant includes about 25 mg/ml phospholipids (including from about 11.0 mg/ml to about 15.5 mg/ml disaturated phosphatidylcholine), from about 0.5 mg/ml to about 1.75 mg/ml triglycerides, from about 1.4 mg/ml to about 3.5 mg/ml of free fatty acids, and no more than 1.0 mg/ml, and preferably no more than 0.25 mg/ml of lung surfactant proteins. Such components may be obtained from a natural lung extract, such as bovine lung extract. These components are suspended in a 0.9% saline solution. The proteins are lung surfactant protein B and lung surfactant protein C. An example of such a surfactant is sold by Ross Laboratories (Columbus, Ohio) under the trademark Survanta®, and is described further in the Physicians' Desk Reference, Volume 47, pgs. 2069-2071 (1993).

In another embodiment, the lipid-containing surfactant includes from about 50 wt. % to about 100 wt. % dipalmitoyl phosphatidylcholine, preferably from about 50 wt. % to about 80 wt. %, from 0 wt. % to about 40 wt. % palmitoyloleoylphosphatidylglycerol, preferably from about 10 wt. % to about 20 wt. %, and from 0 wt. % to about 20 wt. % palmitic acid, preferably from about 5 wt. % to about 10 wt. %. Most preferably, the dipalmitoyl phosphatidylcholine is present in the surfactant in an amount of about 69 wt. %, the palmitoyloleoylphosphatidylglycerol is present in the surfactant in an amount of about 22 wt. %, and the palmitic acid is present in the surfactant in an amount of about 9 wt. %.

In another embodiment, the surfactant is a perfluorocarbon-containing surfactant. In general, such surfactants include a perfluorocarbon having from 3 to 12 carbon atoms. Such perfluorocarbons may include acid functional groups (*e.g.*, carboxyl groups) or functional groups including positively charged metal ions at one or both ends of the perfluorocarbon chain.

Representative examples of perfluorocarbon-containing surfactants which may be employed include, but are not limited to, perfluorodecalin (Green Cross Corp., Japan), and surfactants including perfluorocarbons having 8 carbon atoms and which are sold under the trade names FC75 and FC77 (3M, St. Paul, Minnesota). It is to be understood, however, that the scope of the present invention is not to be limited to any particular perfluorocarbon surfactant.

Applicants have found that, when lung cells are contacted with genetic material and a surfactant, that the surfactant does not inactivate the genetic material, that the surfactant eliminates the air-fluid interface in the lung, and that the surfactant provides for enhanced distribution of the genetic material throughout the lung

tissue; *i.e.*, genetic material administered to lung tissue in combination with a surfactant transduces cells throughout the lung and the respiratory tract. The use of the surfactant enables the genetic material to transduce lung parenchymal cells as well as cells in the trachea and bronchus. The use of the surfactant also favors expression of transduced genetic material in lung parenchymal cells over expression of such genetic material in the large airways (*i.e.*, trachea, vocal cords, carinii, and bronchus).

In one embodiment, the surfactant may be present in an amount of from about 0.5 mg/ml to about 25 mg/ml, preferably from about 5 mg/ml to about 25 mg/ml.

Genetic material which may be contained in the expression vehicle which is introduced into the lung cells includes, but is not limited to, polynucleotides or nucleic acid sequences of DNA or RNA (including antisense oligonucleotides). Such polynucleotides or nucleic acid sequences may encode a therapeutic agent. The term "therapeutic" is used in a generic sense and includes treating agents, prophylactic agents, and replacement agents.

The term "nucleic acid sequence" as used herein, means a DNA or RNA molecule, and more particularly a linear series of deoxyribonucleotides or ribonucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of the adjacent pentoses. Depending upon the use herein, such term includes complete and partial gene sequences, and includes polynucleotides as well.

Expression vehicles which may be introduced into the lung cells include, but are not limited to, plasmid vectors, such as, for example, prokaryotic plasmid vectors (*e.g.*, bacterial expression vectors), eukaryotic plasmid vectors (*e.g.*, yeast vectors and fungal vectors), and viral plasmid vectors, including retroviral plasmid vectors,

adenoviral plasmid vectors, and adeno-associated virus plasmid vectors. When the expression vehicle is a viral plasmid vector, such plasmid vector may be contained within an infectious viral vector particle, such as a retroviral vector particle, an adenoviral vector particle, or an adeno-associated virus vector particle.

In one embodiment, the expression vehicle is a viral vector particle, sometimes hereinafter referred to as a "viral vector." The viral vector may be a retroviral vector, an adenoviral vector, an adeno-associated virus vector, or a Herpes Virus vector.

In one embodiment, the viral vector is an adenoviral vector.

The adenoviral vector which is employed may, in one embodiment, be an adenoviral vector which includes essentially the complete adenoviral genome (Shenk, *et al.*, Curr. Top. Microbiol. Immunol., 111(3): 1-39 (1984)). Alternatively, the adenoviral vector may be a modified adenoviral vector in which at least a portion of the adenoviral genome has been deleted.

In one embodiment, the adenoviral vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; and at least one DNA sequence encoding a therapeutic agent. The vector is free of at least the majority of adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter.

In still another embodiment, the gene in the E2a region that encodes the 72 kilodalton binding protein is mutated to produce a temperature sensitive protein that is active at 32°C, the temperature at which the viral particles are produced, but is inactive at 37°C, the temperature of the animal or human host. This temperature sensitive mutant is described in Ensinger, *et al.*, J.

Virology, 10:328-339 (1972), Van der Vliet, *et al.*, J. Virology, 15:348-354 (1975), and Friefeld, *et al.*, Virology, 124:380-389 (1983).

Such a vector, in a preferred embodiment, is constructed first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the 5' end, the "critical left end elements," which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a multiple cloning site; a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The vector also may contain a tripartite leader sequence. The DNA segment corresponding to the adenoviral genome serves as a substrate for homologous recombination with a modified or mutated adenovirus, and such sequence may encompass, for example, a segment of the adenovirus 5 genome no longer than from base 3329 to base 6246 of the genome. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. Representative examples of such shuttle plasmids include pAvS6, shown in Figure 4. The DNA encoding the therapeutic agent then may be inserted into the multiple cloning site. One may amplify the expression of the DNA encoding the therapeutic agent by adding to the plasmid increased copies of the DNA encoding the therapeutic agent.

This construct is then used to produce an adenoviral vector. Homologous recombination is effected with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted. Such homologous recombination may be effected through co-transfection of the plasmid vector and the modified adenovirus into a helper cell line, such as 293 (embryonic kidney epithelial) cells (ATCC No. CRL

1573), by $CaPO_4$ precipitation. The helper cells may be contacted with the plasmid vector, the modified adenovirus, and the surfactant, which aids in enabling the plasmid vector and modified adenovirus to transfect the helper cells. Upon such homologous recombination, a recombinant adenoviral vector is formed that includes DNA sequences derived from the shuttle plasmid between the NotI site and the homologous recombination fragment, and DNA derived from the E1 and E3 deleted adenovirus between the homologous recombination fragment and the 3' ITR.

In one embodiment, the homologous recombination fragment overlaps with nucleotides 3329 to 6246 of the adenovirus 5 (ATCC VR-5) genome.

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; at least the DNA sequence which encodes a therapeutic agent; a poly A signal; adenoviral DNA free of at least the majority of the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. The vector also may include a tripartite leader sequence. This vector may then be transfected into a helper cell line, such as the 293 helper cell line, which will include the E1a and E1b DNA sequences, which are necessary for viral replication, and to generate infectious adenoviral particles. The surfactant, which may be as hereinabove described, may be used to aid in enabling the vector to be transfected into the helper cell line.

The infectious adenoviral vector particles may be administered *in vivo* to lung cells of a host in combination with the surfactant, whereby the infectious adenoviral vector particles will infect lung cells *in vivo* in a host, thereby providing for *in vivo* expression of the therapeutic agent in the host. The host may be an animal host, which includes mammalian hosts. The mammalian host may be a

human or non-human primate host. The surfactant may be present in a concentration of from about 0.5 mg/ml to about 25 mg/ml, preferably from about 5 mg/ml to about 25 mg/ml. The viral particles are administered in an amount effective to produce a therapeutic effect in a host. In one embodiment, the adenoviral vector particles may be administered in an amount of at least 1 plaque forming unit, and in general such amount does not exceed 10^{14} plaque forming units, and preferably such amount is from about 10^5 to about 10^{13} pfu, more preferably from about 10^5 to about 10^{11} pfu, and most preferably from about 1.5×10^8 to about 1×10^{11} pfu. The exact dosage of adenoviral vector particles which may be administered is dependent upon a variety of factors including the age, sex, and weight of the patient, the therapeutic agent which is to be administered, and the severity of the disorder to be treated.

The infectious adenoviral vector particles and the surfactant may be administered to the lung cells by intranasal, intratracheal, or endotracheal administration. The infectious adenoviral vector particles and the surfactant also may be administered in the form of an aerosol.

The adenoviral vector particles and the surfactant may be administered in combination with a pharmacologically acceptable carrier or solution. Such pharmaceutical carriers or solutions, include, but are not limited to, saline solution, water, and aqueous buffers such as phosphate buffers and Tris buffers. The selection of a suitable pharmaceutical carrier or solution is deemed to be apparent to those skilled in the art from the teachings contained herein.

The adenoviral vector particles include DNA encoding at least one therapeutic agent for treating or preventing a disease or disorder of the lung. DNA sequences encoding

such a therapeutic agent, and which may be placed into the adenoviral vector include, but are not limited to, the CFTR (cystic fibrosis transmembrane conductance regulator) gene; and DNA encoding lung surfactant proteins such as surfactant protein A, surfactant protein B, surfactant protein C, and surfactant protein D; the α-1 antitrypsin gene; genes that encode receptors that bind to cytokines; the GM- CSF gene; and genes encoding interleukins such as Interleukin-1 and Interleukin-2; and genes encoding the Interleukin-1 and Interleukin-2 receptors.

Other DNA sequences encoding therapeutic agents which may be included in the adenoviral vector particles include, but are not limited to, genes encoding antioxidants such as Cu-Zn-SOD and Mn-SOD; catalase genes; the dominant negative PDGF gene; and genes encoding clotting factors such as Factor VIII and Factor IX.

The DNA sequence encoding the at least one therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; lung surfactant protein promoters, such as the SP-A, SP-B and SP-C promoters; the CCSP promoter, the TTF-1 promoter; or other lung epithelial cell specific promoters; the β-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the therapeutic agent. It is to be understood, however, that the scope of the

present invention is not to be limited to specific foreign genes or promoters.

The present invention is particularly applicable to the treatment of cystic fibrosis. For example, infectious adenoviral particles including the CFTR gene may be administered, in combination with the surfactant, to a patient in the amounts hereinabove mentioned. The surfactant will enable the adenoviral particles to be distributed throughout the respiratory system, including the trachea, vocal cords, carinii, bronchus, bronchioles, alveoli, and lung parenchymal cells. Because the adenoviral vector particles are distributed throughout the respiratory system, the adenoviral vector particles containing the CFTR gene transduce cells located throughout the respiratory system, whereby the CFTR gene is expressed in lung parenchymal cells as well as cells of the trachea and bronchus.

Other lung diseases or disorders which may be treated include, but are not limited to, respiratory distress syndrome caused by lung surfactant protein deficiency states, whereby adenoviral vector particles including a gene encoding a lung surfactant protein gene (such as surfactant protein A, surfactant protein B, surfactant protein C or surfactant protein D) are administered to a patient in combination with a surfactant; lung fibrosis caused by α-1 antitrypsin deficiency, whereby adenoviral vector particles including the α-1-antitrypsin gene are administered to the patient in combination with the surfactant; and lung inflammation, which may be treated by administering to a patient adenoviral vector particles including one or more genes encoding receptors which bind to cytokines, in combination with the surfactant. It is to be understood, however, that the scope of the present invention is not to be limited to the treatment of any particular disease or disorder of the lung.

In addition, the method of the present invention may be employed in animal models for the treatment of diseases or disorders of the lung. In such an animal model, animal models may be administered combinations of adenoviral vector particles including a gene encoding a therapeutic agent for treating a disease or disorder of the lung, and the surfactant, in varying dosages and amounts. Through the use of such animal models, one can determine the amounts of adenoviral vector particles and of surfactant which are effective in treating a disease or disorder of the lung in a patient.

The method of the present invention also may be employed to transduce lung cells *in vitro* with an adenoviral vector including a DNA sequence encoding a therapeutic agent. The transduced cells then may be cultured *in vitro*, and then administered to a patient for treatment of a disease or disorder of the lung.

In another alternative, lung cells may be contacted *in vitro* with adenoviral vector particles including a gene encoding a therapeutic agent for the treatment of a disease or disorder of the lung and the surfactant. Upon such transduction of the lung cells, the lung cells produce the therapeutic agent *in vitro*, whereby such therapeutic agent may be obtained from the culture of lung cells and administered to a patient.

In another embodiment, the viral vector is a retroviral vector.

Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. Preferably, the retroviral vector is an infectious but non-replication competent

retrovirus; however, replication competent retroviruses may also be used.

In one embodiment, the retroviral vector may be a Moloney Murine Leukemia Virus of the LN series of vectors, such as those hereinabove mentioned, and described further in Bender, *et al.*, J. Virol., Vol. 61 , pgs. 1639-1649 (1987) and Miller, *et al.*, Biotechniques, Vol. 7, pgs. 980-990 (1989). Such vectors have a portion of the packaging signal derived from a mouse sarcoma virus, and a mutated gag initiation codon. The term "mutated" as used herein means that the gag initiation codon has been deleted or altered such that the gag protein or fragments or truncations thereof, are not expressed.

In another embodiment, the retroviral vector may include at least four cloning, or restriction enzyme recognition sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; *i.e.*, the restriction product has an average DNA size of at least 10,000 base pairs. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI. In a preferred embodiment, the retroviral vector includes each of these cloning sites. Such vectors are further described in U.S. Patent Application Serial No. 08/340,805, filed November 17, 1994, and incorporated herein by reference in its entirety.

When a retroviral vector including such cloning sites is employed, there may also be provided a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral vector. The shuttle cloning vector also includes at least one desired gene which is capable of being transferred from the shuttle cloning vector to the retroviral vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible, or complementary cloning sites hereinabove described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art.

The shuttle cloning vector can be employed to amplify DNA sequences in prokaryotic systems. The shuttle cloning vector may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322; pUC 18; etc.

The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and ß-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The vector then is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψCRE, ψCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990), which is incorporated herein by

reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the therapeutic agent(s). Such retroviral vector particles then may be employed, in the presence of the surfactant, to transduce lung cells *in vivo*. The transduced lung cells will express the nucleic acid sequence(s) encoding the therapeutic agent(s). The surfactant may be present at concentrations such as those hereinabove described.

The retroviral vector particles may transduce the lung cells *in vivo* at a titer of from 10^6 to 10^{12} infectious units per ml, preferably from 10^6 to 10^7 infectious units per ml.

Therapeutic agents which may be encoded by at least one nucleic acid sequence contained in the viral vector particles may be those as hereinabove described. The vector also may include an antisense DNA or RNA sequence. Promoters controlling such nucleic acid sequences also may be those hereinabove described.

In one embodiment, the retroviral vector particles may include a negative selective marker or "suicide" gene, such as a viral thymidine kinase gene, such as the Herpes Simplex Virus thymidine kinase gene, the cytomegalovirus thymidine kinase gene, and the varicella-zoster virus thymidine kinase gene. The retroviral vector particles then may be administered, in combination with the surfactant, *in vivo*, to lung cancer cells, whereby the retroviral vector particles including the negative selective marker transduce the lung cancer cells. The

patient then is given an interaction agent, such as, for example, gancyclovir or acyclovir, whereby the transduced lung cancer cells are killed.

The retroviral vector particles including a gene encoding a therapeutic agent for the treatment of a disease or disorder of the lung, may be administered in combination with the surfactant in varying amounts to animals in animal models for the treatment of diseases or disorders of the lung. Also, the retroviral vector particles and the surfactant may be administered to lung cells *in vitro* for the *in vitro* production of therapeutic agents for the treatment of diseases or disorders of the lung.

The combination of genetic material, which may be contained in expression vehicles as hereinabove described, and a surfactant (in particular a lipid-containing surfactant such as those hereinabove described) may be employed in transducing other eukaryotic cells *in vivo* or *ex vivo* or *in vitro* in addition to lung cells. Such eukaryotic cells include, but are not limited to, epithelial cells of the nasal and ear passages. Other eukaryotic cells which may be transduced include, but are not limited to, bone marrow cells, hepatocytes, and fibroblasts.

EXAMPLES

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Construction of Av1Luc1

A. Construction of pAvS6

The adenoviral construction shuttle plasmid pAvS6 was constructed in several steps using standard cloning techniques including polymerase chain reaction based cloning techniques. First, the 2913 bp BglII, HindIII fragment was removed from Ad-dl327 and inserted as a blunt

fragment into the XhoI site of pBluescript II KS- (Stratagene, La Jolla, CA) (Figure 1).

Ad-dl327 is identical to adenovirus 5 except that an XbaI fragment including bases 28591 to 30474 (or map units 78.5 to 84.7) of the Adenovirus 5 genome, and which is located in the E3 region, has been deleted. The E3 deletion in Ad-dl327 is similar to the E3 deletion in Ad-dl324, which is described in Thimmappaya *et al.*, Cell, 31:543 (1983). The complete Adenovirus 5 genome is registered as Genbank accession #M73260, incorporated herein by reference, and the virus is available from the American Type Culture Collection, Rockville, Maryland, U.S.A. under accession number VR-5.

Ad-dl327 was constructed by routine methods from Adenovirus 5 (Ad5). The method is outlined briefly as follows and previously described by Jones and Shenk, Cell, 13:181-188, (1978). Ad5 DNA is isolated by proteolytic digestion of the virion and partially cleaved with Xba I restriction endonuclease. The Xba I fragments are then reassembled by ligation as a mixture of fragments. This results in some ligated genomes with a sequence similar to Ad5, except excluding sequences 28591 bp to 30474 bp. This DNA is then transfected into suitable cells (*e.g.* KB cells, HeLa cells, 293 cells) and overlaid with soft agar to allow plaque formation. Individual plaques are then isolated, amplified, and screened for the absence of the 1878 bp E3 region Xba I fragment.

The orientation of this fragment was such that the BglII site was nearest the T7 RNA polymerase site of pBluescript II KS$^-$ and the HindIII site was nearest the T3 RNA polymerase site of pBluescript II KS$^-$. This plasmid was designated pHR. (Figure 1).

Second, the ITR, encapsidation signal, Rous Sarcoma Virus promoter, the adenoviral tripartite leader (TPL) sequence and linking sequences were assembled as a block

using PCR amplification (Figure 2). The ITR and encapsidation signal (sequences 1-392 of Ad-dl327 [identical to sequences from Ad5, Genbank accession #M73260] incorporated herein by reference) were amplified (amplification 1) together from Ad-dl327 using primers containing NotI or AscI restriction sites. The Rous Sarcoma Virus LTR promoter was amplified (amplification 2) from the plasmid pRC/RSV (sequences 209 to 605; Invitrogen, San Diego, CA) using primers containing an AscI site and an SfiI site. DNA products from amplifications 1 and 2 were joined using the "overlap" PCR method (amplification 3) (Horton *et al.*, BioTechniques, 8:528-535 (1990)) with only the NotI primer and the SfiI primer. Complementarity between the AscI- containing end of each initial DNA amplification product from reactions 1 and 2 allowed joining of these two pieces during amplification. Next the TPL was amplified (amplification 4) (sequences 6049 to 9730 of Ad-dl327 [identical to similar sequences from Ad5, Genbank accession #M73260]) from cDNA made from mRNA isolated from 293 cells (ATCC Accession No. CRL 1573) infected for 16 hrs. with Ad-dl327 using primers containing SfiI and XbaI sites respectively. DNA fragments from amplification reactions 3 and 4 were then joined using PCR (amplification 5) with the NotI and XbaI primers, thus creating the complete gene block.

Third, the ITR-encapsidation signal-TPL fragment was then purified, cleaved with NotI and XbaI and inserted into the NotI, XbaI cleaved pHR plasmid. This plasmid was designated pAvS6A- and the orientation was such that the NotI site of the fragment was next to the T7 RNA polymerase site (Figure 3).

Fourth, the SV40 early polyA signal was removed from SV40 DNA as an HpaI-BamHI fragment, treated with T4 DNA polymerase and inserted into the SalI site of the plasmid pAvS6A- (Figure 3) to create pAvS6 (Figures 3 and 4).

B. Construction of Av1Luc1

Av1Luc1 (Figure 7) (Yei, *et al.*, Gene Therapy, Vol. 1, pgs. 192-200 (1994)), an E1-deleted (1.18 map units to 9.2 map units), E3-deleted (78.5 map units to 84.7 map units) adenoviral vector is constructed first by inserting the firefly luciferase gene (Genbank Accession No. M15077) into the EcoRV site of pAvS6 so that the 5' end of the firefly luciferase gene was closest to the Adenovirus 5 tripartite leader. The firefly luciferase gene was obtained from pGEM-luc (Figure 5 - Promega). pGEM-luc was digested with StuI and HindIII in order to splice out the firefly luciferase gene.

The firefly luciferase gene was inserted into the EcoRV site of pAvS6 so that the 5' end of the firefly luciferase coding sequence was closest to the Adenovirus 5 tripartite leader. The resulting plasmid, pAvS6-Luc1 (Figure 6) was linearized with KpnI and recombined with the large (35 kb) ClaI fragment of Ad-dl327 in 293 cells as described in Trapnell, Advanced Drug Delivery Reviews, Vol. 12, pgs. 185-199 (1993) to form Av1Luc1 (Figure 7). The viral vector then was propagated, purified by double-banding in CsCl gradients, and titered in 293 cells as described in Rosenfeld, *et al.*, Cell, Vol. 68, pgs. 143-155 (1992).

Example 2

Each rabbit was treated with one of four different doses of Av1Luc1 by tracheal instillation. The virus was suspended in 2 ml phosphate buffered saline or 10 mg/ml of Survanta® surfactant, which is an organic solvent extract of minced bovine lung containing the materials hereinabove described. Five rabbits each received 1.5×10^8 pfu of Av1Luc1 suspended in saline or in Survanta® surfactant. Four rabbits each received 6.25×10^8 pfu of Av1Luc1 suspended in saline or in Survanta® surfactant. Five rabbits each received 2.5×10^9 pfu of Av1Luc1 suspended in

saline or in Survanta® surfactant. Five rabbits each received 1 x 10^{10} pfu of Av1Luc1 suspended in saline or in Survanta® surfactant. Three days after administration of the adenovirus, the rabbits were killed, and the lungs from each group of rabbits were assayed for luciferase activity in all lung tissue as well as in the large airways (*i.e.*, trachea, vocal cords, carinii, and bronchus) and parenchyma. For the processing and the luciferase assay of lung and large airway tissue conducted in all experiments of this example, a lysis buffer, a reaction buffer, and a luciferin solution were prepared. The lysis buffer contained 1% Triton X-100 octylphenoxy polyethoxyethanol surfactant (250 µl of 100% stock), 25 mM glycylglycine (2.5 ml of 0.25M stock) (pH7.8), 15 mM $MgSO_4$ (375 µl of 1M stock), 4 mM EDTA (200 µl of 0.5M stock), 1 mM dithiothreitol (DTT) (25 µl of 1M stock), and 21.65 ml of dd (doubly distilled) H_2O per 25 ml of buffer. The reaction buffer contained 25 mM glycylglycine (0.8 ml of 2.25M stock), 15 mM $MgSO_4$ (120 µl of 1M stock), 4 mM EDTA (64 µl of 0.5M stock), 15 mM potassium phosphate (120 µl of 1M stock) (pH7.8), 1 mM DTT (8 µl of 1M stock), 2 mM ATP (0.8 ml of 20 mM stock), and 6.09 ml of dd H_2O per 8 ml of buffer. The luciferin solution contained 0.4 mM luciferin (2.4 ml of 1 mM stock), 25 mM glycylglycine (0.6 ml of 0.25M stock), 15 mM $MgSO_4$ (90 µl of 1M stock), 4 mM EDTA (48 µl of 0.5M stock), 2 mM DTT (12 µl of 1M stock), and 2.85 ml of dd H_2O per 6 ml of solution.

Lung and large airway tissues were processed and assayed as follows:

Tissue from the right and left lungs of each group of rabbits was placed in a 250 ml bottle containing 25 ml lysis buffer. Tissue from the trachea, vocal cords, carinii, and bronchus of each group of rabbits is placed in a 50 ml centrifuge tube containing 2 ml of lysis buffer. All tissue samples then were frozen in the lysis buffer at

-20°C until such samples were assayed for luciferase activity.

Prior to the assay, the samples were thawed and diluted with lysis buffer such that there was 0.1 g of tissue per 3 ml of lysis buffer. Each sample then was homogenized with a large probe for 30 to 45 seconds. Duplicate aliquots of 100 μl of each sample then were pipetted into a tube. Control tubes contained 100 μl of lysis buffer alone, 100 μl of a pooled lung sample containing a low amount of luciferase, and 100 μl of a pooled lung sample containing 10^6 light units of luciferase. 360 μl of reaction buffer then was added to each tube. The assay tubes were shaken gently, and 100 μl of the luciferin solution then was added to each well. The samples then were read in a luminometer, and luciferase activity for each sample was determined. As shown in Figures 8, 9, and 10, Survanta surfactant increased total lung expression, lung parenchyma expression, and trachea and bronchus expression over 4-fold at the lower viral doses. As shown in Figure 11, the total percentage of expression in the trachea and bronchus was lower in the rabbits treated with surfactant at the higher doses of adenovirus as compared with the rabbits treated with PBS at the higher doses of adenovirus. Virus titers did not change with incubation in either saline or 10 mg/ml surfactant.

In another experiment, each rabbit in groups of 4 or 5 rabbits was given 1 x 10^9 pfu of the adenovirus hereinabove described in combination with Survanta® surfactant in doses ranging from 0.5 mg/ml to 25 mg/ml, or in combination with a saline solution (Control). Five rabbits received PBS; five rabbits received 0.5 mg/ml of surfactant; five rabbits received 2 mg/ml of surfactant; four rabbits received 10 mg/ml of surfactant; and five rabbits received 25 mg/ml of surfactant. Three days after administration of the

adenovirus, the rabbits were sacrificed and the lungs were assayed for luciferase activity in the large airways and parenchyma. As shown in Figure 12, total expression of luciferase in the lungs increased about 4-fold with surfactant concentrations of 10 mg/ml and 25 mg/ml relative to rabbits receiving the virus in saline suspension. The percent of total luciferase expression in the trachea and bronchus also decreased significantly from 43 ± 13% to less than 10% with surfactant concentrations greater than 2 mg/ml.

The above results indicate that the surfactant can enhance adenoviral mediated gene transfer and expression in lung tissue. In addition, the use of surfactant provides for an increase in the proportion adenoviral mediated gene transfer and expression in lung parenchyma cells vis-a-vis large airway expression.

In another experiment, each rabbit was given 1 x 10^9 pfu of Av1Luc1 suspended in 2 ml of phosphate buffered saline or 10 mg/ml of Survanta® surfactant. Three days after administration of the adenovirus, five rabbits that were given PBS and four rabbits that were given surfactant were sacrificed, and the lungs were assayed for luciferase activity. Seven days after administration of the adenovirus, five rabbits that were given PBS and five rabbits that were given surfactant were sacrificed, and the lungs were assayed for luciferase activity. Fourteen days after administration of the adenovirus, five rabbits that were given PBS and five rabbits that were given surfactant were sacrificed, and the lungs were assayed for luciferase activity. As shown in Figure 13, at 3 and 7 days after adenoviral administration, increased luciferase expression was found in the rabbits that were given the surfactant as compared with those rabbits that were given phosphate buffered saline.

Example 3

Adenoviral vector. The adenoviral vector employed in this example is Av1luc1 as hereinabove described.

Preparation of Radioactive Adenoviral Vector. Radioactively labeled adenovirus vector was prepared by metabolic-labeling during vector amplification in 293 cells. Cell monolayers were cultured in plastic roller bottles (1500 cm^2 surface area) in Dulbecco's Modified Eagles Medium (DMEM; Gibco BRL) supplemented with 10% fetal bovine serum, 10 mM Hepes pH 7.0 at 37°C. When cells were 90% confluent, the media was removed and replaced with 30 ml of infection medium: phosphate-free DMEM containing the adenovirus vector (multiplicity of infection = 5 pfu/cell) supplemented with 2% fetal bovine serum, 0.1% D-glucose, 10 mM Hepes, pH 7.0 containing ^{33}P-orthophosphate (0.5 mCi, 1000-3000 Ci/mmol, Amersham). Infection was continued for 4 hours at which time 70 ml of infection medium without additional vector or ^{33}P label was added. Twelve hours after infection, an additional 100 ml of infection medium was added and incubation continued for a total of 50 hours. Amplified adenovirus vector was then isolated, purified and titered as previously described (Mittereder *et al.*, Human Gene Therapy, Vol. 5, pgs. 717-729 (1994)). The specific activity of the radio-labeled vector was determined by liquid scintillation counting to be 1.28×10^{-4} cpm/plaque forming unit. ^{33}P radioactivity was >98% trichloroacetic acid precipitable before and after aerosolization of the vector.

Surfactant-adenoviral suspensions. The surfactant employed was Survanta® as hereinabove described. All dilutions of surfactant and adenovirus were with sterile buffered saline (0.14 M NaCl, 0.27 M KCl, and 0.01 M phosphate buffer at pH 7.4). The adenovirus was kept frozen at -70°C until thawed and mixed with saline or 10 mg/ml surfactant in saline.

Aerosolizer. A low-flow prototype gas jet aerosolizer provided by Baxter Labs designed to run at flow rates of 2-4 l/min. was employed. All aerosolizations were begun with 10 ml fluid in the reservoir and were performed at a flow rate of 1.5 l/min. oxygen to optimize the match of aerosolizer output with the minute ventilation of the rabbits as much as possible. The reservoir of the aerosolizer was kept on crushed ice to minimize foaming of surfactant containing suspensions (Lewis *et al.*, J. Appl. Physiol., Vol. 7, pgs. 1270-1276, (1991); Lewis *et al.*, Am. J. Respir. Dis., Vol. 147, pgs. 1364-1370 (1993)). A 15 ml aerosol charging chamber was placed between the proximal end of the endotracheal tube and the ventilator to increase efficiency of deposition (Lewis *et al.*, 1991). To characterize particle size, the output of the aerosolizer was monitored by connecting the aerosolizer loosely to a 6 in. long by 1 in. diameter plastic tube connected in series to a cyclone and then to a 7 stage cascade impactor (Intox Products, Albuquerque, NM). The cyclone and the cascade impactor were designed for particle sampling a flow of 2 l/min. The cyclone is a collection chamber designed to collect particles greater than 6.3 microns mean aerodynamic diameter. Therefore, the output of the aerosolizer was supplemented with 0.5 l/min of gas to achieve the 2 l/min flow through the impaction system. The saline in the reservoir contained in various combinations ^{99}Tc-sulfur colloid, ^{3}H-dipalmitoylphosphatidylcholine labeled surfactant, and/or ^{33}P-adenovirus. ^{99}Tc-sulfur colloid was chosen as a tracer for the aerosol because it is high molecular weight and will not be cleared rapidly from the lungs (Lewis, *et al.*, 1993). About 1mCi ^{99}Tc-sulfur colloid was added to the reservoir. The [choline-methyl^{3}H-] dipalmitoylphosphatidylcholine (30 ci/mmol) (DuPont NEN, Boston, MA) was added to a chloroform:methanol extract of Survanta as a tracer, the radiolabel and Survanta were

resuspended in saline with glass beads, and the mixture was combined with Survanta prior to aerosolization (Lewis *et al.*, 1991). After timed aerosol collection, the collection chamber of the cyclone and the impaction plates were rinsed with saline and aliquots were used to measure radioactivity. The volume of aerosol collected on the impaction plates was estimated based on the radioactivities of aliquots of the suspension in the reservoir of the aerosolizer.

Treatment of rabbits. The treatment protocol was designed to minimize the variability resulting from aerosolizing the vector. All treatments used virus from a single batch and all aerosolizations were conducted over the 2 hour aerosolization period characterized by the initial studies of the aerosolizer. Instillation suspensions also were made from the same vector suspensions, and the instillations were performed concurrently with the aerosolizations. All treatment groups contained 5 or 6 rabbits. Because of concerns about the consistency of delivery of aerosols to the rabbits, the study was designed to monitor aerosol delivery and distribution in alternate rabbits.

On one day, a suspension of 9×10^9 PFU adenovirus/ml that contained ^{99}Tc-sulfur colloid in 11 ml saline was prepared. Ten ml of this suspension was added to the aerosolizer, and 1 ml was diluted with saline such that rabbits treated by instillation received 4×10^8 PFU adenovirus in 4 ml saline. Female New Zealand White rabbits weighing 1.9 ± 0.1 kg were anesthetized with intramuscular ketamine (50 mg/kg) and acepromazine (0.5 mg/kg) and placed in the supine position. Each rabbit was intubated orotracheally with a cuffed endotracheal tube (inner diameter, 3.0 mm) during spontaneous ventilation (Conlon *et al.*, Lab. Animal Sci., Vol. 40, pgs. 221-222 (1990)). The rabbit then was ventilated with a time-cycled

pressure-limited ventilator set to deliver a peak inspiratory pressure of 13 cm H_2O, a positive end expiratory pressure of 1 cm H_2O at a rate of 40 breaths/min with a 0.7 sec inspiratory time and 100% oxygen. The rabbit was given 0.2 mg/kg pancuronium bromide via an ear vein and the tidal volume was measured (Pulmonary Monitor CP-100) (Bicore, Irvine, CA).

Each rabbit was sequentially assigned to receive 5 min of aerosol followed by removal of the lungs for immediate measurement of distribution of ^{99}Tc-sulfur colloid, or 15 min aerosol and recovery for measurement of reporter gene expression 3 days later. The time period of 5 minutes for the distribution measurements was used to conserve the vector-containing aerosol suspension and to minimize the total period of aerosolization. It was assumed that 5 min and 15 min aerosolizations result in the same distributions of the vector and ^{99}Tc sulfur colloid.

During the aerosolizations, other rabbits were anesthetized, ventilated manually with an anesthesia bag with 100% oxygen and given 0.2 mg/kg pancuronium bromide to avoid a cough reflex. Each rabbit was positioned with the right chest dependent and 2 ml of the total 4 ml volume of suspension at 4°C was injected rapidly through a 3.5 Fr catheter at a level 2 cm above the carina, the endotracheal tube was connected to an anesthesia bag for manual ventilation at a rate of 30 breaths per minute with sufficient pressure to move the chest for 30 seconds as the same positioning of the chest was maintained. The rabbit was returned to a midline supine position for 30 seconds. The remaining 2 ml of the viral suspension then was administered with the left chest in the dependent position followed by the same manual ventilation for 30 seconds. The animal was returned to the midline position. Alternate animals were killed within 2 min of instillation and the lungs removed for the measurement of the immediate

distribution of ^{99}Tc-sulfur colloid or recovered for luciferase measurements 3 days after instillation. Neostigmine methylsulfate (0.5 mg/kg) and atropine sulfate (0.04 mg/kg) were given to reverse the effect of pancronium bromide for the rabbits used for the expression studies. All rabbits were killed by intravascular injection with 100 mg/kg pentobarbital.

On a separate day, a parallel experiment was performed using 11 ml of saline containing the same viral titre, 10 mg/ml surfactant and ^{99}Tc-sulfur colloid. Alternate rabbits received aerosol, again for 5 or 15 min and the lungs were used for measurements of ^{99}Tc distribution (5 min exposures) or expression (15 min exposures). Other rabbits received the vector in 4 ml saline containing 10 mg/ml surfactant using the same instillation techniques as described above.

Processing of lungs. The lungs from 5 animals for each study group were removed intact and the lung parenchyma was manually stripped from the proximal airways, leaving the left and right mainstem bronchi and second order bronchi attached to the trachea. The trachea from just proximal to the carina to the larynx was separated and processed separately from the carina plus bronchi. The lung parenchyma was divided into lobes for lobar distribution measurements and for expression measurements. The trachea, and carina plus bronchi were processed separately. All tissue was homogenized with a Techmar homogenizer using 30 times the tissue weight of lysis buffer (1% Triton x 100, 25 mM glycylglycine, 15 mM Mg SO_4, 4 mM EDTA, and 1 mM DTT at pH 7.8).

Luciferase assay. Aliquots of the homogenates in lysis buffer were added to 360 μl of reaction buffer (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EDTA, 1 mM DTT, 2 mM ATP, and 15 mM potassium phosphate at pH 7.8) in glass tubes. After temperature equilibration, 100 μl luciferin reagent (0.4 mM Luciferin, 25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM

EDTA, and 2mM DTT) was injected and light was quantified with the Moonlight 2010 luminometer (San Diego, CA) (Brasier *et al.*, Biotechniques, Vol. 7, pgs. 1116-1122 (1989); Zsengeller *et al.*, Human Gene Therapy, Vol. 6, pgs. 457-467 (1995)). The assay was linear over the tissue luciferase contents encountered in these experiments.

Data analysis. All values are expressed as means ± standard error of the mean. Differences between groups were tested by two tailed Student t tests. Significance was accepted at $p<0.05$.

RESULTS

Characterization of aerosol. The aerosolized vector with and without surfactant was evaluated by sampling the reservoir and measuring particle size distribution with a cascade impactor. The vector was labeled with ^{33}P, the saline was labeled with ^{99}Tc-sulfur colloid, and the surfactant was labeled with ^{3}H-dipalmitoylphosphatidylcholine. Aerosolization for the time period subsequently used for the exposures of rabbits resulted in an increasing relative concentration of the vector and a decreasing concentration of ^{99}Tc-sulfur colloid in the reservoir (Fig. 14A). Aerosolization in the presence of 10 mg/ml surfactant resulted in no significant change in surfactant concentration and less change in vector and ^{99}Tc-sulfur colloid than with aerosolization without surfactant (Fig. 14B). Therefore, the concentrations of components in the reservoir changed with time of aerosolization and surfactant minimized these changes.

The aerosols had proportionate impaction of the vector, ^{99}Tc-sulfur colloid and surfactant on the cascade impactor plates (Fig. 15). This result indicated that there was no fractionation of the different components of the aerosol based on particle size. The ratio of ^{33}P-vector to ^{99}Tc-sulfur colloid averaged 2.3 ± 0.2 indicating preferential aerosolization of the vector relative to ^{99}Tc-sulfur colloid. This result was the opposite of what was anticipated because the ^{33}P-vector was concentrated in the reservoir and the ^{99}Tc-sulfur colloid was lost. Presumably ^{99}Tc-sulfur colloid was lost to the aerosolization system, perhaps by loss to plastic surfaces. It was not concentrated in the aerosolized particles. In contrast the ratios for ^{33}P-vector and ^{3}H-surfactant to ^{99}Tc-sulfur colloid in aerosol recovered on the impaction plates were 1.22 ± 0.04 and 0.97 ± 0.004, respectively. Therefore,

surfactant minimized the aberrant behavior of the ^{99m}Tc-sulfur colloid in the aerosol. The volume of aerosol recovered on the impaction plates was estimated by the average ^{99}Tc-sulfur colloid concentration measured in the reservoir just before and after the collection period. The volume aerosolized per 15 min time interval did not change over the 120 min aerosolization periods studied for the two mixtures. The aerosolized volume estimated by ^{33}P-vector collection was 131 ± 9 μl in the absence of surfactant and 86 ± 4.3 μl in the presence of surfactant ($p<0.05$). Although the volume aerosolized in the presence of surfactant was less, this result needs to be viewed with caution because of the approximations that enter into the volume calculation. For example, a volume calculation based on ^{99}Tc-sulfur colloid yielded a higher volume for the aerosol with surfactant.

Treatment of rabbits. The rabbits used for the measurements of ^{99}Tc-sulfur colloid distributions had blood drawn for arterial blood gas and pH values at the end of the 5 min aerosolization period. There were no differences between the 5 rabbits receiving aerosol without surfactant and the 5 rabbits receiving aerosol with surfactant. The mean values were: pH 7.38 ± 0.01, pO_2 490 ± 15 mmHg and pCO_2 34 ± 2 mmHg. The tidal volumes for the animals in all aerosolized groups ranged from 13 to 16 ml/kg and there were no significant differences between groups. All rabbits recovered from the anesthesia and were healthy at 3 days after vector exposure.

Distribution of aerosol and instillate following treatment. The distributions of aerosolized and instilled ^{99}Tc-sulfur colloid following treatment with the vector without surfactant demonstrated 94% of the label in the parenchyma by instillation and 68% of the label in the parenchyma with aerosolization ($p<0.01$) (Fig. 16). The aerosol preferentially localized to the trachea (25%) and the

carina and bronchi (8%) ($p<0.01$ versus instillation). The same measurements made with vector in surfactant resulted in virtually identical distributions between proximal airways and lung parenchyma.

The suspensions that localized to the lung parenchyma distributed equivalently to the lobes with either aerosolization or instillation, and surfactant did not alter lobar distributions (Fig. 17). The distributions were proportionate to the weights of each lobe. Therefore, there was no preferential distribution based on lobe.

Expression of luciferase. Instilled vector in saline resulted in $1.9 \pm 0.3 \times 10^6$ luciferase light units and vector instilled with surfactant resulted in $4.2 \pm 1.2 \times 10^6$ light units in the total lung ($p = 0.08$). Total expression in the lungs resulting from aerosolized vector was $5.3 \pm 1.2 \times 10^6$ light units without surfactant and $4.8 \pm 1.7 \times 10^6$ light units with surfactant. The efficiencies of expression of aerosol relative to instillation could not be evaluated because of uncertainties about volume of vector aerosolized.

Surfactant did not change the distribution of luciferase activity between the parenchyma, trachea and carina plus bronchi for aerosolized vector (Fig. 18). Surfactant also did not significantly alter the distribution of expression after instillation because of variability in the measurement. However, there was a difference in distributions of expression of luciferase when the aerosolization and instillation techniques were compared. Overall 30 ± 18% of the luciferase expression was in the parenchyma after aerosolization versus 72 ± 8% after instillation ($p<0.001$). Instillation resulted in 24 ± 8% of the expression in the trachea while aerosolization resulted in 66 ± 9% of the expression in the trachea ($p<0.01$).

A comparison of the initial distributions of the vector containing solutions as measured by ^{99}Tc-sulfur colloid with the distributions of luciferase expression demonstrated decreased percent expression in lung parenchyma. For the animals receiving vector by instillation, the parenchymal recovery of ^{99}Tc-sulfur colloid was 94 ± 1% while 72 ± 8% of the luciferace activity was in the parenchyma ($p<0.01$). The absolute differences were larger after aerosol delivery of the vector. Immediately after aerosolization, 68 ± 2% of the aerosol was found in the lung parenchyma, and only 30 ± 8% of luciferase expression was in the parenchyma ($p<0.01$).

A goal of this example was to explore some of the variables that may influence the efficacy of the aerosol delivery of vectors for gene therapy to the lungs. Adenovirus was selected as the vector because of its ability to efficiently transfect the airway epithelium of the lung (Rosenfeld *et al.*, Cell, Vol. 68, pgs. 143-155 (1992); Yei *et al.*, Gene Ther., Vol. 1, pgs. 192-200 (1994)). The combination of the adenovirus vector and surfactant also was studied because surfactant can increase the rate of spreading and improve the distribution of saline suspensions instilled into the lungs (Kharasch, *et al.*, Am. Rev. Respir. Dis., Vol. 144, pgs. 909-913 (1992)). It was found that there was not a proportional retention of the radiolabeled vector and ^{99}Tc-sulfur colloid in the reservoir of the aerosolizer with time of aerosolization. In the absence of surfactant, the vector was concentrated and the sulfur-colloid was lost. The presence of 10 mg/ml surfactant resulted in a much more proportional aerosolization of three component ^{99}Tc-sulfur-colloid, surfactant, and adenovirus suspensions. ^{99}Tc-sulfur colloid was reported previously to not be aerosolized proportionately to pentamidine (O'Riordan *et al.*, Am. Rev. Respir. Dis., Vol. 145, pgs. 1117-1122 (1992)). It was

found in preliminary experiments that the smaller molecular weight ^{99}Tc-DTPA was aerosolized proportionately to the water phase, but this low molecular weight compound is cleared rapidly from the lungs (Wollmer *et al.*, Surfactant Therapy for Lung Diesease, Robertson *et al.*, eds., pgs. 199-213 Marcel Dekker, New York (1995)).

It was also determined whether the aerosolized adenovirus was fractionated selectively into particles of different sizes. Since location of impaction in the lung depends critically on particle size (Raabe, Occupational Lung Diseases, Gee, *et al.*, eds., Raven Press, New York (1984)), gene targeting with aerosolized vectors requires that the vector be present in the particle size of interest. The low-flow jet aerosolizer that was used was working at just below the ideal flow rate because it was desired to reach a compromise between aerosolizer efficiency and the minute ventilation of the animals of about 1060 ml/min (1.9 kg x 40 breaths/min x 14 ml/kg tidal volume) to optimize the efficiency of aerosol delivery to the lungs. The aerosolizer delivered a bimodal particle size profile with primary particle size cut-off diameters of about 5 and 2 microns, and the ^{33}P-adenovirus distributed proportionately to the other radiolabels. There was no effect of surfactant on particle size distribution. In other studies we have found equivalent particle size distributions with ^{99}Tc-DTPA and ^{99}Tc-sulfur colloid. Therefore, the adenovirus vector distributed proportionately to the mass of the particles within each size range. Also titered was the adenovirus after aerosolization and recovery from the plates of the cascade impactor in a preliminary experiment using 293 cells (Mittereder, *et al.*, 1994). Infectivity was not lost and did not change with particle size.

The preferential distribution of the instilled suspensions relative to the aerosolized suspensions in

rabbit lungs resulted from an instillation technique designed to optimize distribution (Jobe, Surfactant Therapy for Lung Disease, 1995). A relatively large volume of suspension quickly was instilled with positioning and ventilation in animals that were paralyzed to avoid cough. This technique resulted in 94% of the instillate being distributed beyond the larger bronchi and uniformly to the lung lobes. In general, instillation of small volumes results in primarily airway localization (Davis, *et al.*, Pediatr. Res., Vol. 31, pgs. 445-450 (1992); Mastrangeli, *et al.*, J. Clin. Invest., Vol. 91, pgs. 225-234 (1993)). As anticipated, more of the aerosol was found on the large airways immediately after aerosolization. The 5 micron particles that comprised about 15-25% of the mass of the aerosol likely would tend to impact on the larger airways (Raabe, 1984). Nevertheless, about 70% of the mass of the aerosol reached the distal lung, and the lobar distribution was equivalent to the instilled suspensions. Surfactant in the instillate or aerosol had no effect on the distributions measured with ^{99}Tc-sulfur colloid. In contrast to previous reports (Davis *et al.*, 1992), the lack of a surfactant effect on lobar distribution resulted from the instillation technique used. Surfactant effects on aerosol distributions in lungs have not been evaluated previously, and no effects were found in these experiments.

The total expression achieved with aerosolization was approximately equivalent to that achieved by installation based on estimates of volume of aerosol delivered to the lungs.

The disclosures of all patents, publications (including published patent applications), database accession numbers, and depository accession numbers referenced in this specification are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication,

database accession number, and depository accession number were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may practiced other than as particularly described and still be within the scope of the accompanying claims.

WHAT IS CLAIMED IS:

1. A process for introducing an expression vehicle into lung cells comprising:

contacting said lung cells with said expression vehicle and at least one surfactant.

2. The process of Claim 1 wherein said at least one surfactant is a lipid-containing surfactant.

3. The process of Claim 2 wherein said contacting is effected with a surfactant composition comprising at least one phospholipid, at least one neutral lipid, at least one fatty acid, and at least one lung surfactant protein selected from the group consisting of surfactant protein B and surfactant protein C.

4. The process of Claim 3 wherein said surfactant composition includes:

(a) about 25 mg/ml phospholipids, in which disaturated phosphatidylcholine is present in an amount of from about 11.0 mg/ml to about 15.5 mg/ml;

(b) from about 0.5 mg/ml to about 1.75 mg/ml of triglycerides;

(c) from about 1.4 mg/ml to about 3.5 mg/ml of free fatty acids; and

(d) surfactant protein B and surfactant protein C in a combined amount not exceeding 1.0 mg/ml.

5. The process of Claim 2 wherein said lipid-containing surfactant includes from about 50 wt.% to about 100 wt.% dipalmitoyl phosphatidylcholine, from about 0 wt.% to about 40 wt.% palmitoyloleoylphosphatidylglycerol, and from about 0 wt.% to about 20 wt.% palmitic acid.

6. The process of Claim 5 wherein said dipalmitoyl phosphatidylcholine is present in said surfactant in an amount of about 69 wt. %, said palmitoyloleoyl-phosphatidylglycerol is present in said surfactant in an amount of about 22 wt.%, and said palmitic acid is present in said lipid-containing surfactant in an amount of about 9 wt.%.

7. The process of Claim 1 wherein said surfactant is present in an amount of from about 5 mg/ml to about 25 mg/ml.

8. The process of Claim 1 wherein said expression vehicle is a plasmid vector.

9. The process of Claim 1 wherein said expression vehicle is an adenoviral vector particle.

10. The process of Claim 1 wherein said expression vehicle is a retroviral vector particle.

11. The process of Claim 1 wherein said lung cells are contacted with said expression vehicle and said surfactant *in vivo*.

12. The process of Claim 1 wherein said lung cells are contacted with said expression vehicle and said surfactant *in vitro*.

13. The process of Claim 1 wherein said expression vehicle includes at least one nucleic acid sequence encoding a therapeutic agent.

14. The process of Claim 1 wherein said at least one surfactant is a perfluorocarbon-containing surfactant.

15. A composition comprising an expression vehicle and at least one lipid-containing surfactant.

16. The composition of Claim 15 wherein said at least one lipid-containing surfactant includes at least one phospholipid, at least one neutral lipid, at least one fatty acid, and at least one lung surfactant protein selected from the group consisting of surfactant protein B and surfactant protein C.

17. The composition of Claim 16 wherein said lipid-containing surfactant includes:

(a) about 25 mg/ml phospholipids, in which disaturated phosphatidylcholine is present in an amount of from about 11.0 mg/ml to about 15.5 mg/ml;

(b) from about 0.5 mg/ml to about 1.75 mg/ml of triglycerides;

(c) from about 1.4 mg/ml to about 3.5 mg/ml of free fatty acids; and

(d) surfactant protein B and surfactant protein C in a combined amount not exceeding 1.0 mg/ml.

18. The composition of Claim 15 wherein said lipid-containing surfactant includes from about 50 wt.% to about 100 wt.% dipalmitoyl phosphatidylcholine, from about 0 wt.% to about 40 wt. % palmitoyloleoyl-phosphatidylglycerol, and from about 0 wt.% to about 20 wt.% palmitic acid.

19. The composition of Claim 18 wherein said dipalmitoyl phosphatidylcholine is present in said lipid-containing surfactant in an amount of about 69 wt.%, said palmitoyloleoylphosphatidylglycerol is present in said lipid-containing surfactant in an amount of about 22 wt.%, and said palmitic acid is present in said lipid-containing surfactant in an amount of about 9 wt.%.

20. The composition of Claim 15 wherein said lipid-containing surfactant is present in said composition in an amount of from about 5 mg/ml to about 25 mg/ml.

21. The composition of Claim 15 wherein said expression vehicle is a plasmid vector.

22. The composition of Claim 15 wherein said expression vehicle is an adenoviral vector particle.

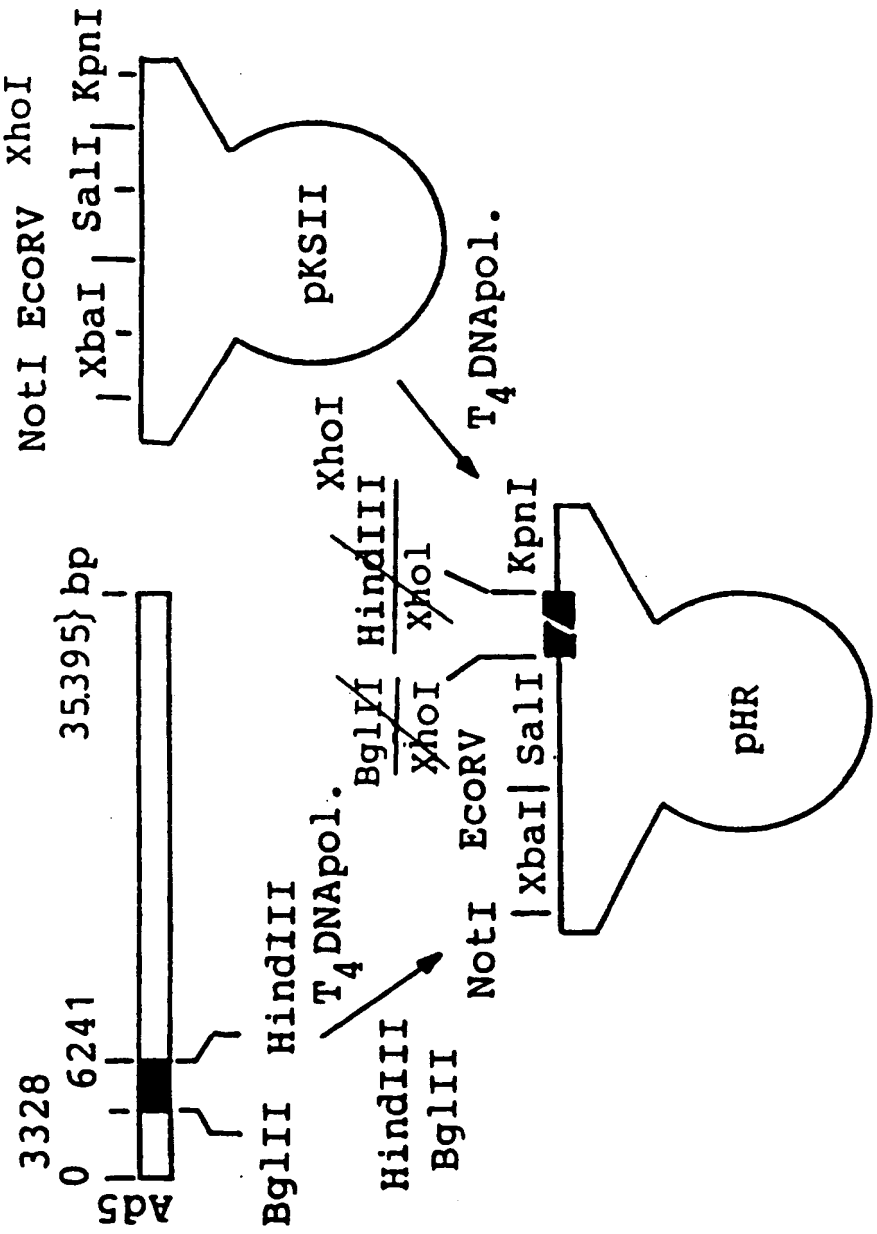
23. The composition of Claim 15 wherein said expression vehicle is a retroviral vector particle.

24. The composition of Claim 15 wherein said expression vehicle includes at least one nucleic acid sequence encoding a therapeutic agent.

25. The composition of Claim 15 and further comprising a pharmaceutically acceptable carrier or solution.

26. The composition of Claim 9 wherein said adenoviral vector particle and said surfactant are administered as an aerosol.

FIG. 1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HindIII
XhoI
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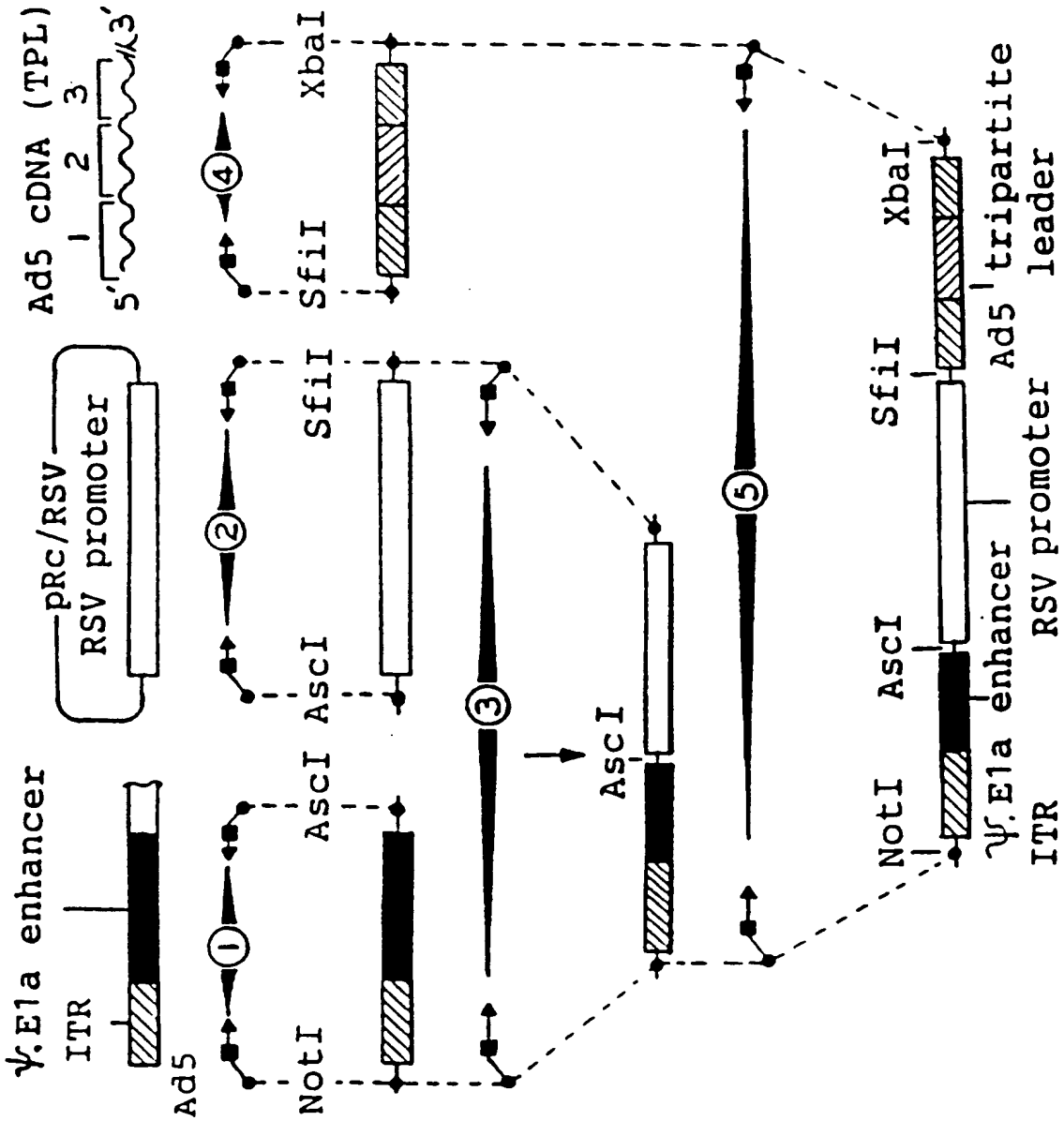
FIG. 2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5' 3'
pRc/RSV
RSV promoter
ψ.E1a enhancer
ITR
Ad5
NotI
AscI
SfiI
XbaI
AscI
SfiI
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SfiI
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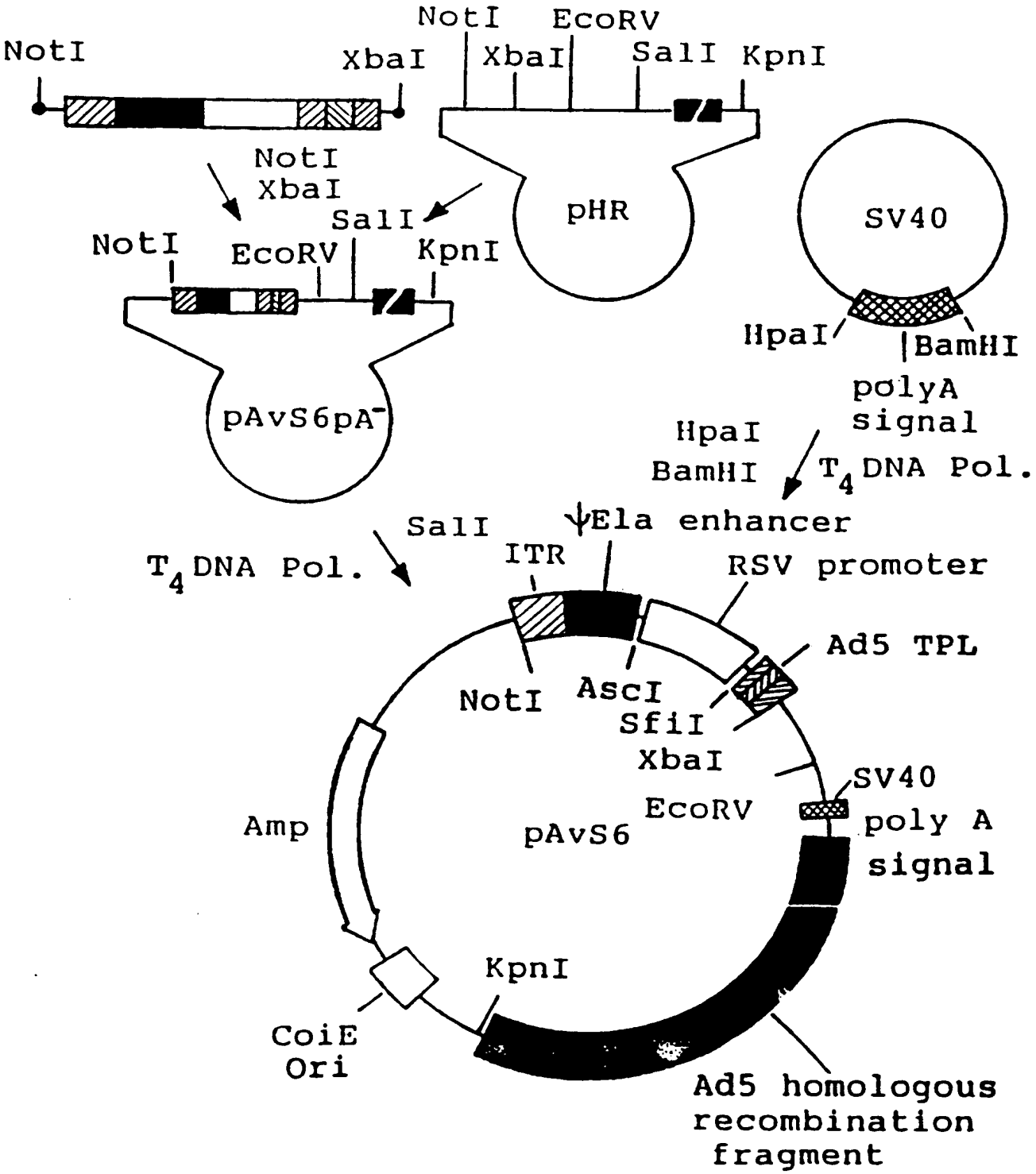
ITR
RSV promoter
1
2
3
4
5

FIG. 3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XbaI
NotI
EcoRV
XbaI
SalI
KpnI
pHR
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XbaI
SalI
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EcoRV
KpnI
pAvS6pA⁻
SV40
HpaI
BamHI
polyA
signal
HpaI
BamHI
T4 DNA Pol.
SalI
T4 DNA Pol.
ψEla enhancer
ITR
RSV promoter
Ad5 TPL
NotI
AscI
SfiI
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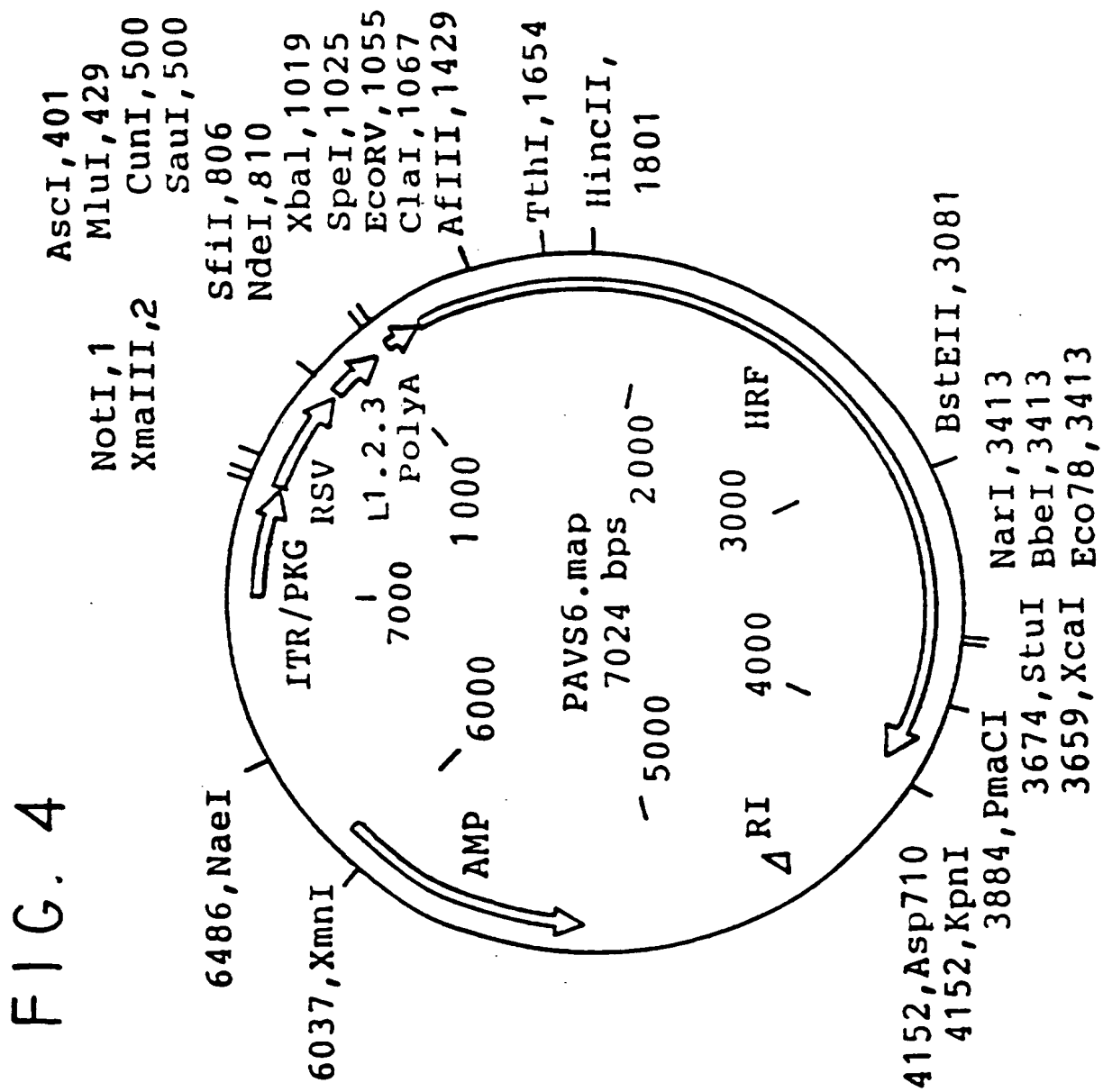
KpnI
CoiE
Ori
Ad5 homologous
recombination
fragment

FIG. 4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SauI,500
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ClaI,1067
AflII,1429
TthI,1654
HincII,
1801
BstEII,3081
NarI,3413
BbeI,3413
Eco78,3413
PmaCI
3674,StuI
3659,XcaI
4152,Asp710
4152,KpnI
3884,
6486,NaeI
6037,XmnI
NotI,1
XmaIII,2
ITR/PKG
RSV
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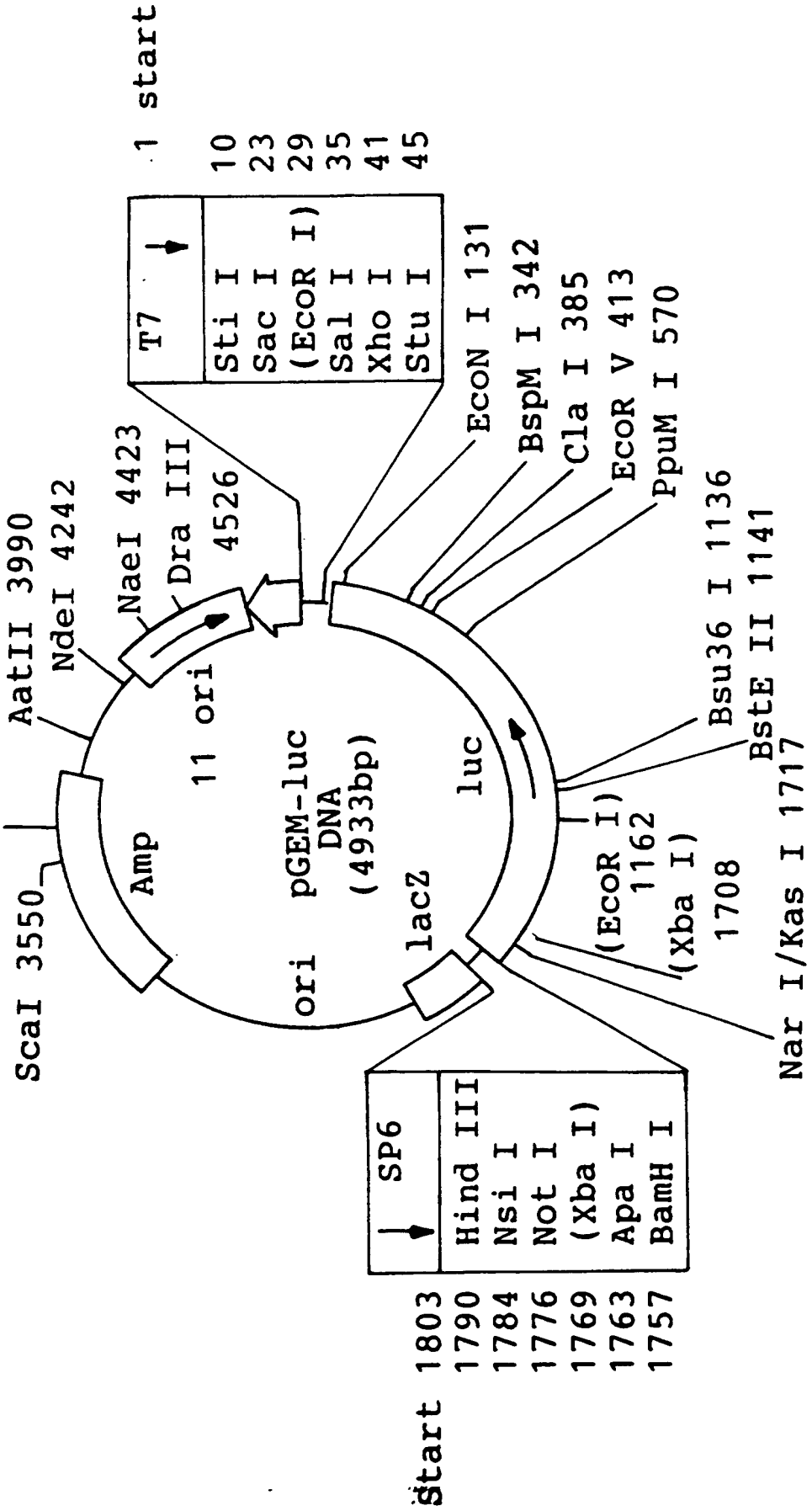
FIG. 5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T7
Sti I 10
Sac I 23
(EcoR I) 29
Sal I 35
Xho I 41
Stu I 45
EcoN I 131
BspM I 342
Cla I 385
EcoR V 413
PpuM I 570
Bsu36 I 1136
BstE II 1141
Nar I/Kas I 1717
(EcoR I) 1162
(Xba I) 1708
SP6
Hind III 1790
Nsi I 1784
Not I 1776
(Xba I) 1769
Apa I 1763
BamH I 1757
Start 1803
ScaI 3550
XmnI 3667
AatII 3990
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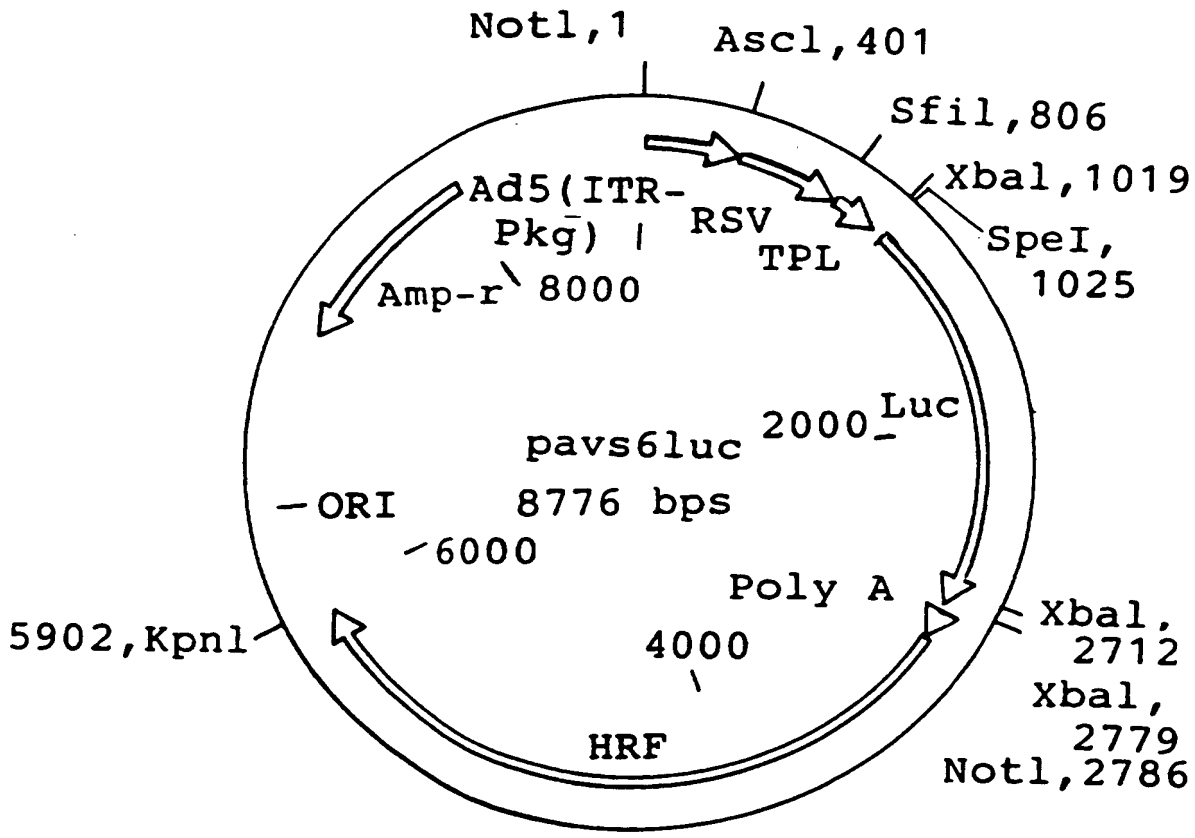
lacZ
luc

FIG. 6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Luc
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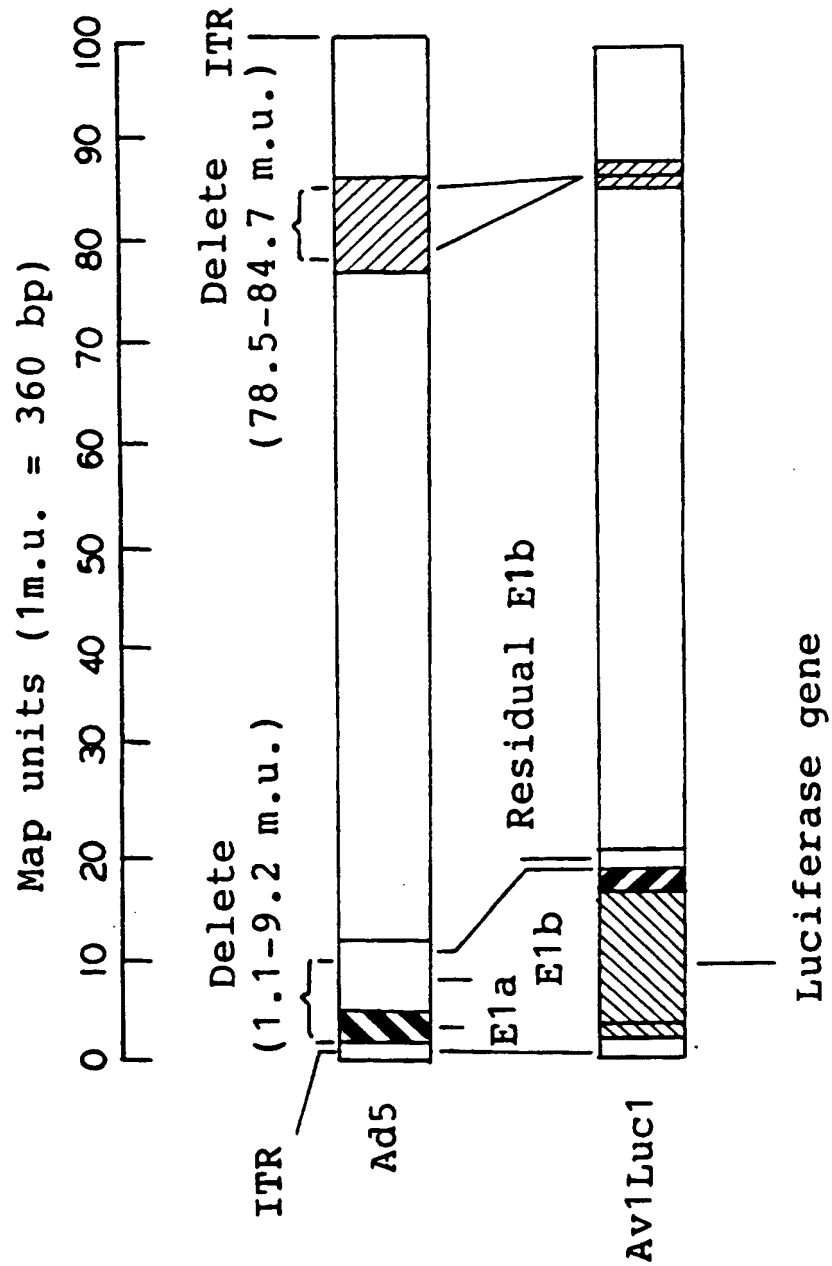
FIG. 7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10
20
30
40
50
60
70
80
90
100
Delete (1.1-9.2 m.u.)
Delete ITR (78.5-84.7 m.u.)
ITR
Ad5
E1a
E1b
Residual E1b
Av1Luc1
Luciferase gene

FIG. 8

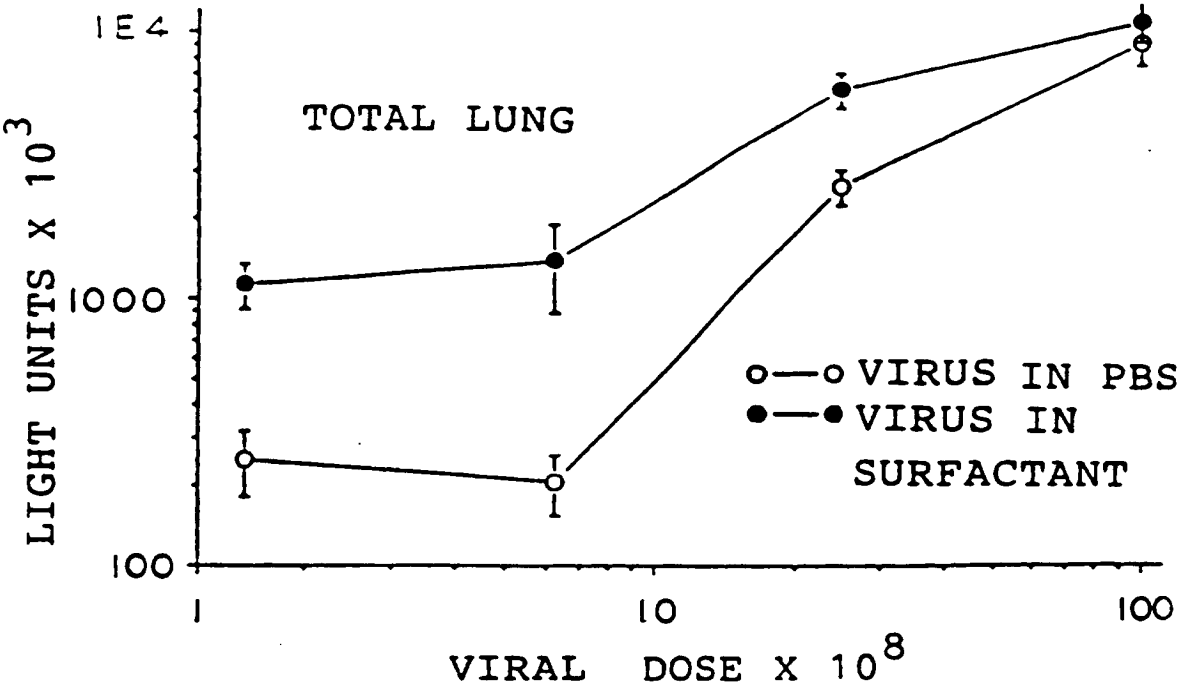

1E4
1000
100
LIGHT UNITS X 10^3
TOTAL LUNG
o—o VIRUS IN PBS
●—● VIRUS IN SURFACTANT
1
10
100
VIRAL DOSE X 10^8

FIG. 9

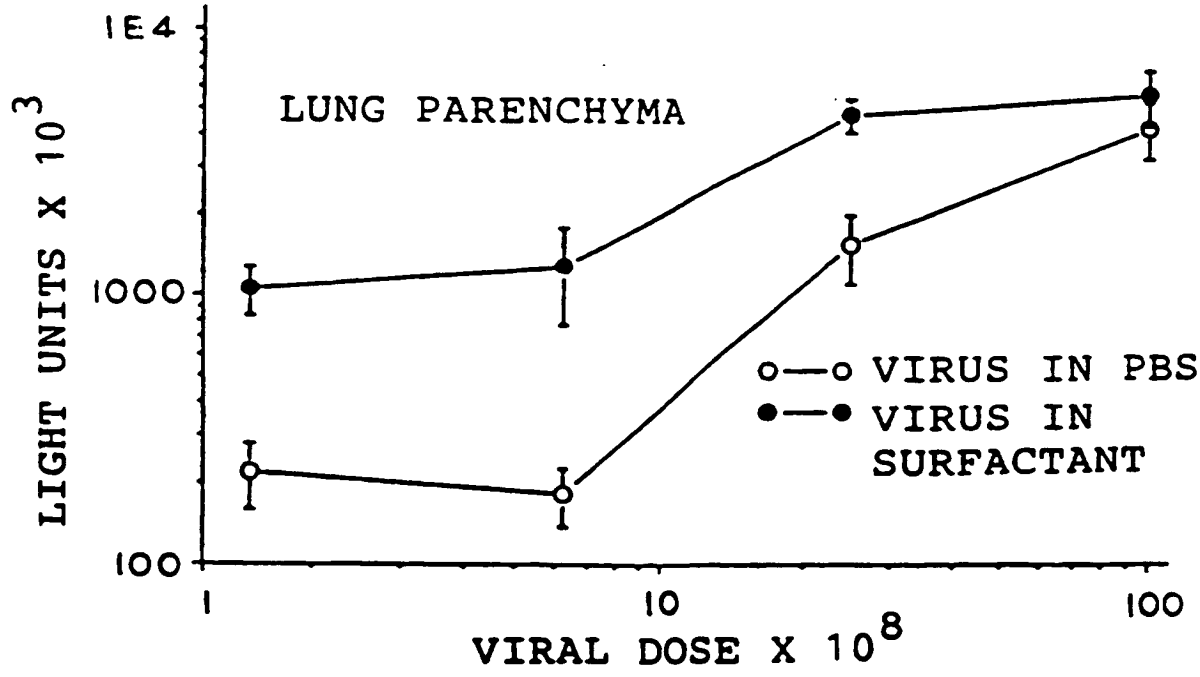

1E4
1000
100
LIGHT UNITS X 10^3
LUNG PARENCHYMA
o—o VIRUS IN PBS
●—● VIRUS IN SURFACTANT
1
10
100
VIRAL DOSE X 10^8

FIG. 10

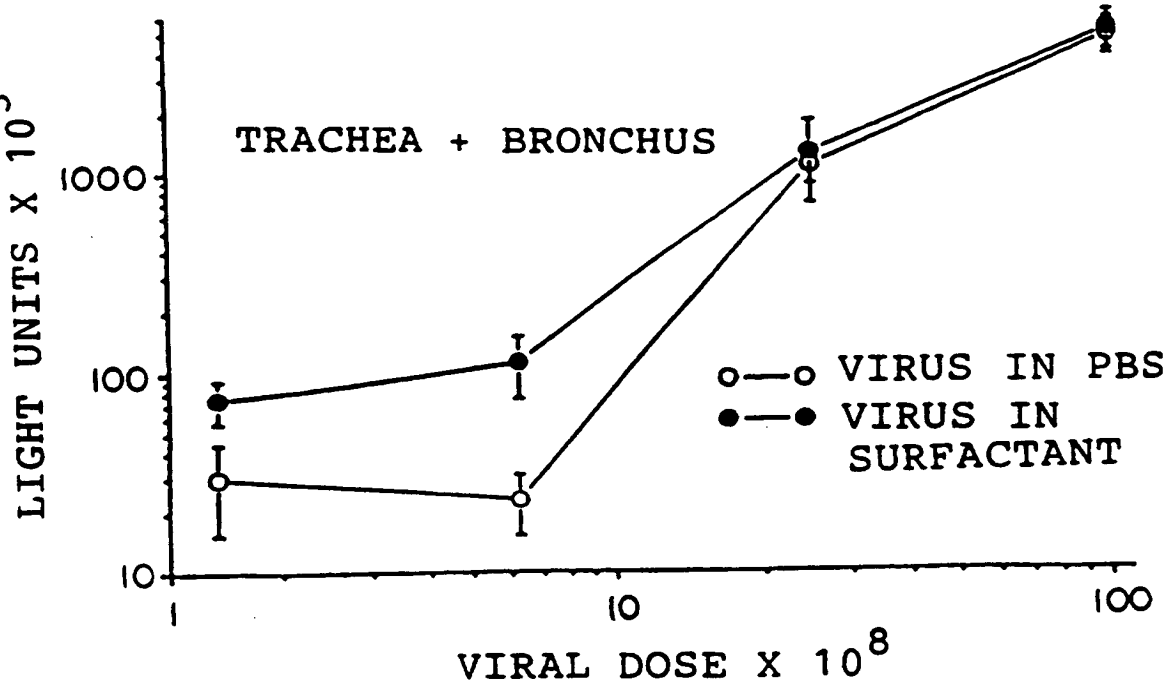

TRACHEA + BRONCHUS
LIGHT UNITS X 10^3
1000
100
10
1
10
100
VIRAL DOSE X 10^8
o—o VIRUS IN PBS
●—● VIRUS IN SURFACTANT

FIG. 11

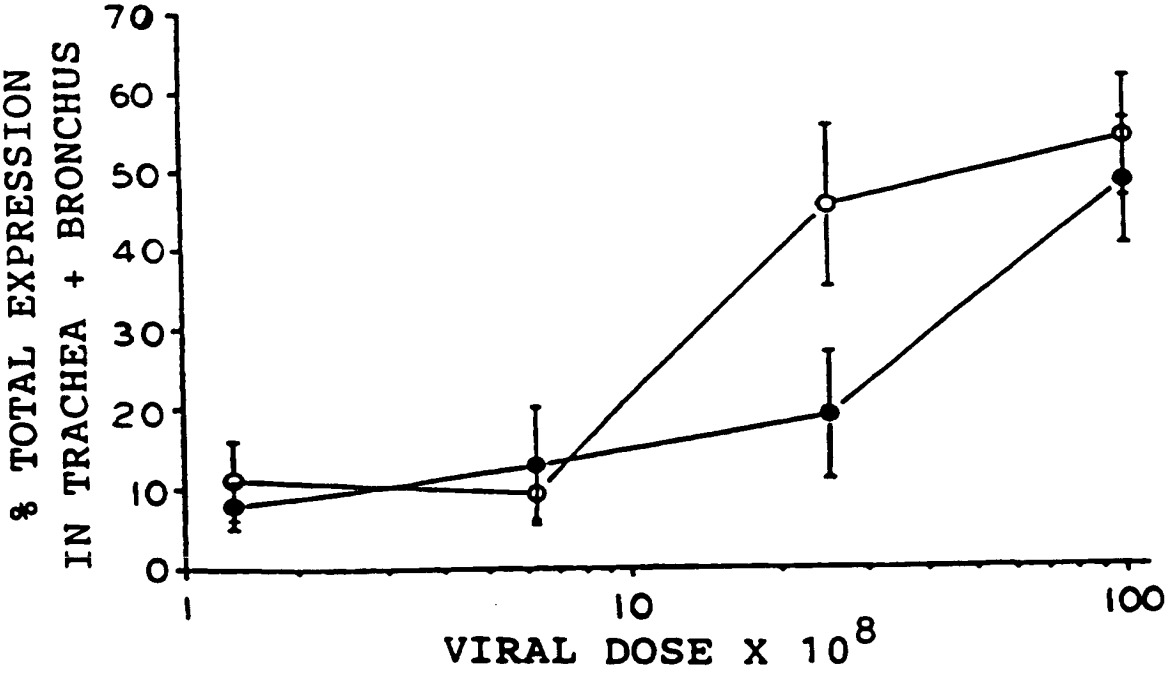

% TOTAL EXPRESSION IN TRACHEA + BRONCHUS
70
60
50
40
30
20
10
0
1
10
100
VIRAL DOSE X 10^8

FIG. 12

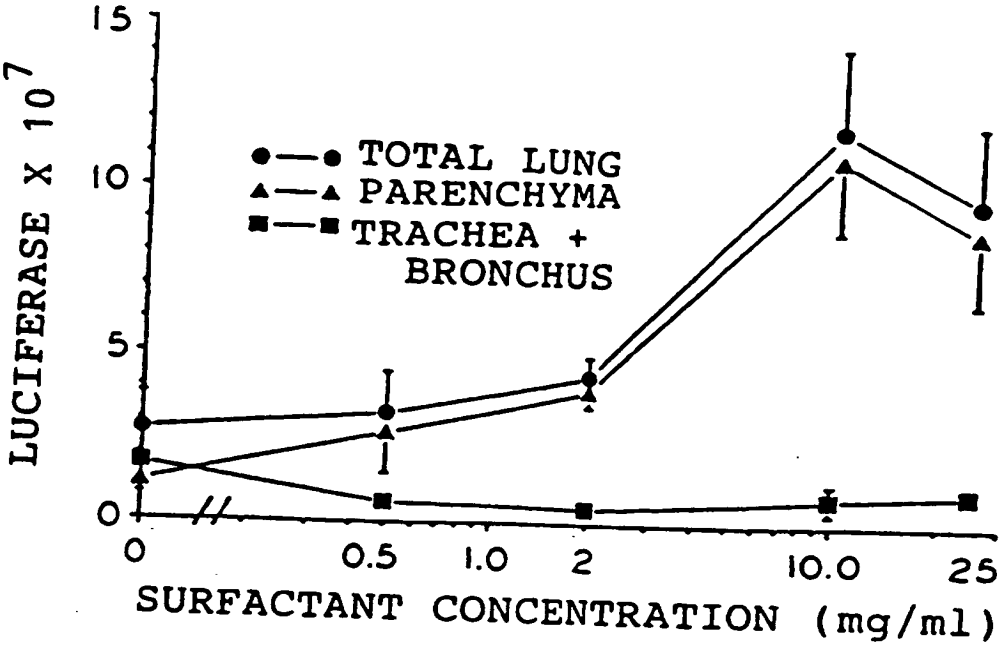

LUCIFERASE X 10^7
15
10
5
0
TOTAL LUNG
PARENCHYMA
TRACHEA + BRONCHUS
0
0.5
1.0
2
10.0
25
SURFACTANT CONCENTRATION (mg/ml)

FIG. 13

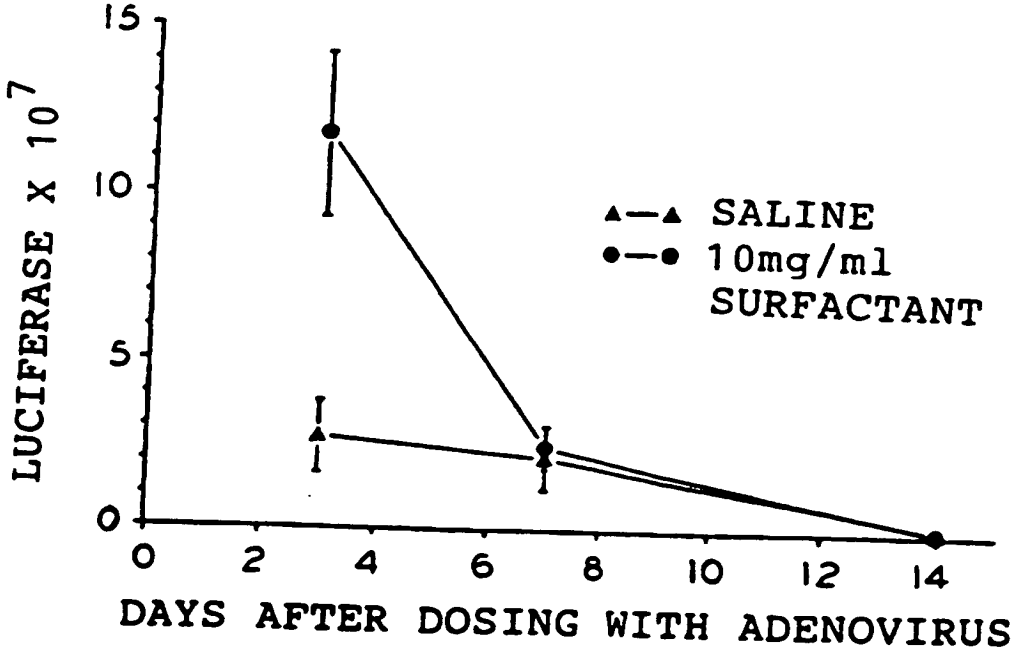

LUCIFERASE X 10^7
15
10
5
0
SALINE
10mg/ml SURFACTANT
0
2
4
6
8
10
12
14
DAYS AFTER DOSING WITH ADENOVIRUS

FIG. 14A

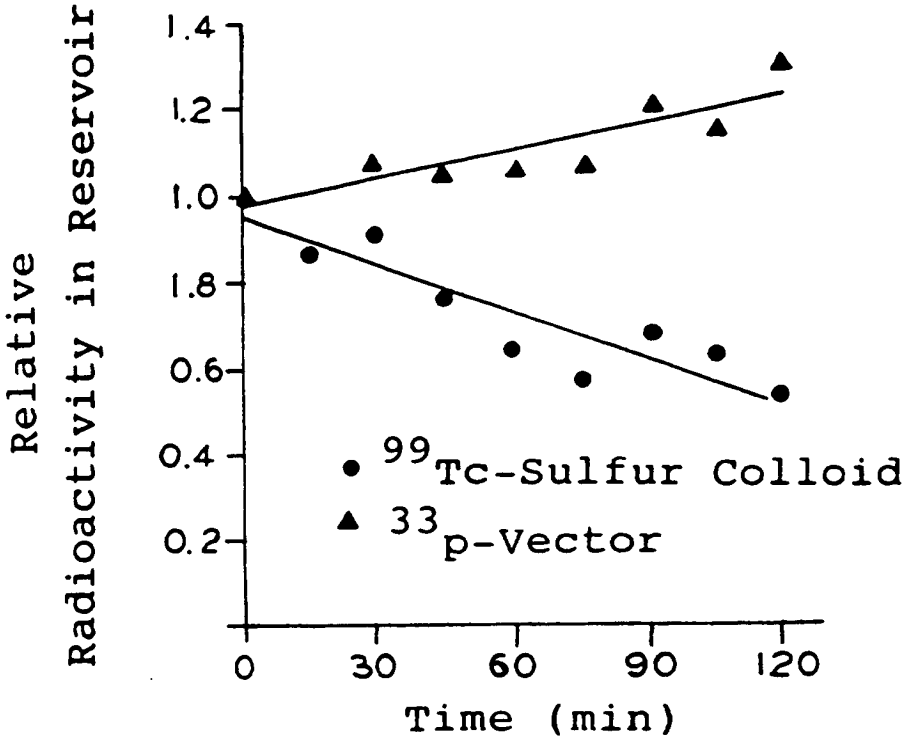


FIG. 14B

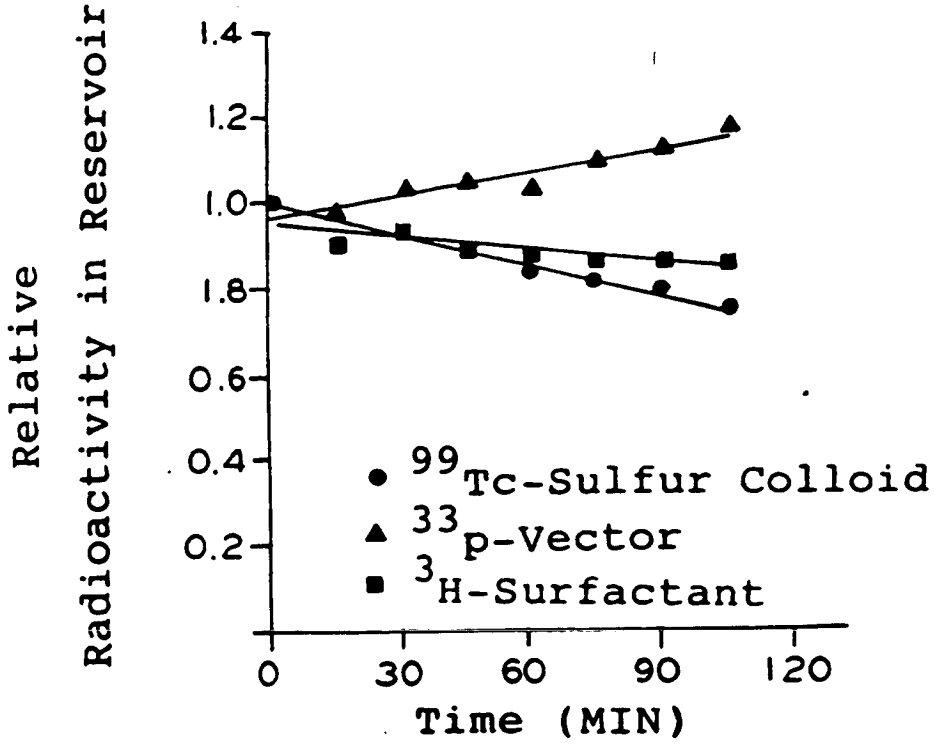
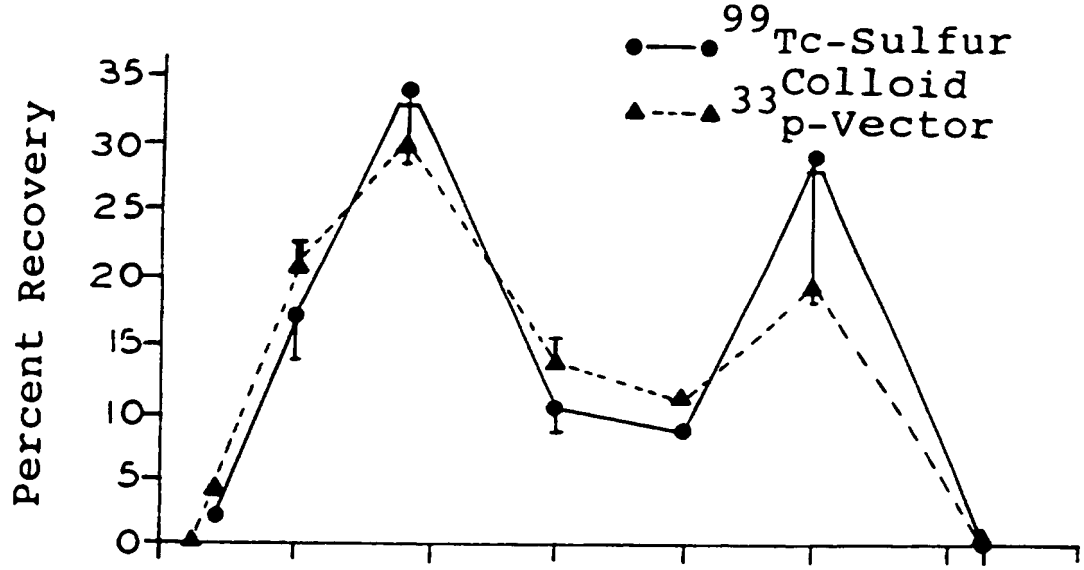


FIG. 15A



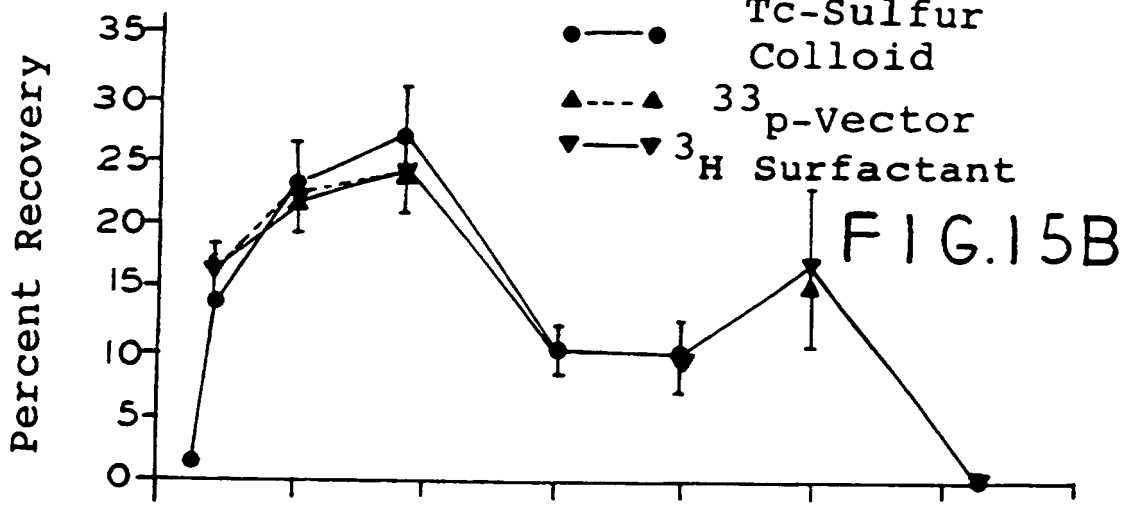


FIG. 15B

FIG. 15C

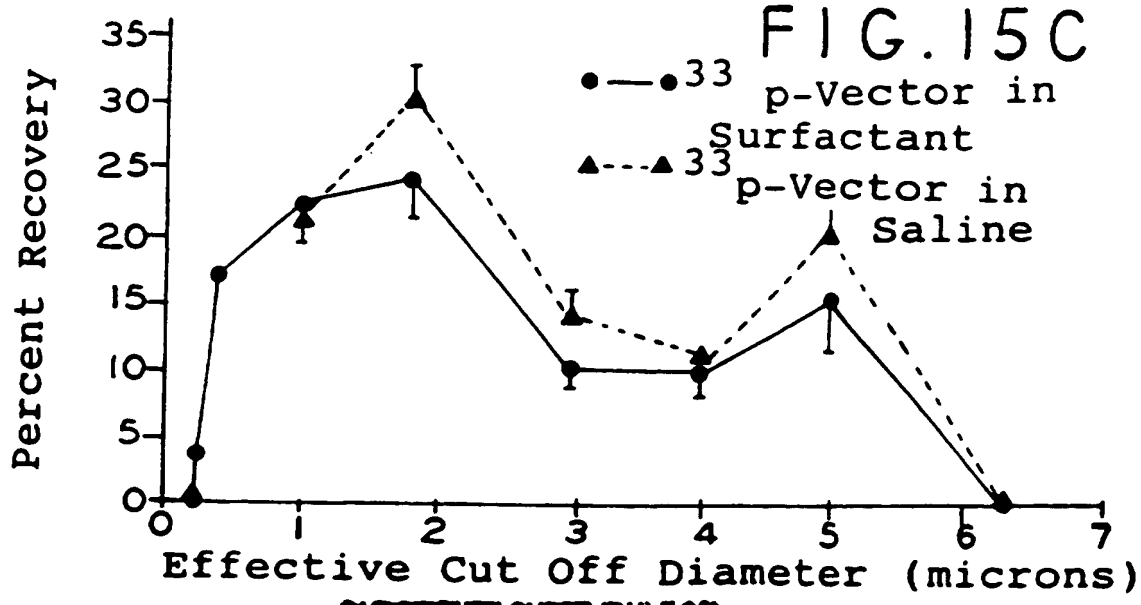


FIG. 16A

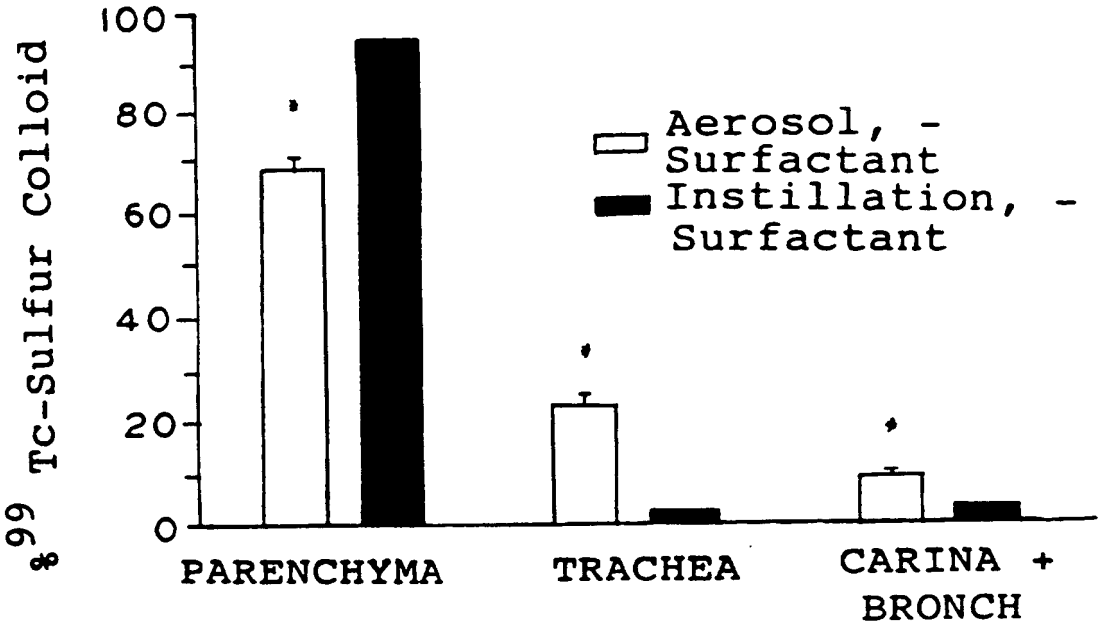


FIG. 16B

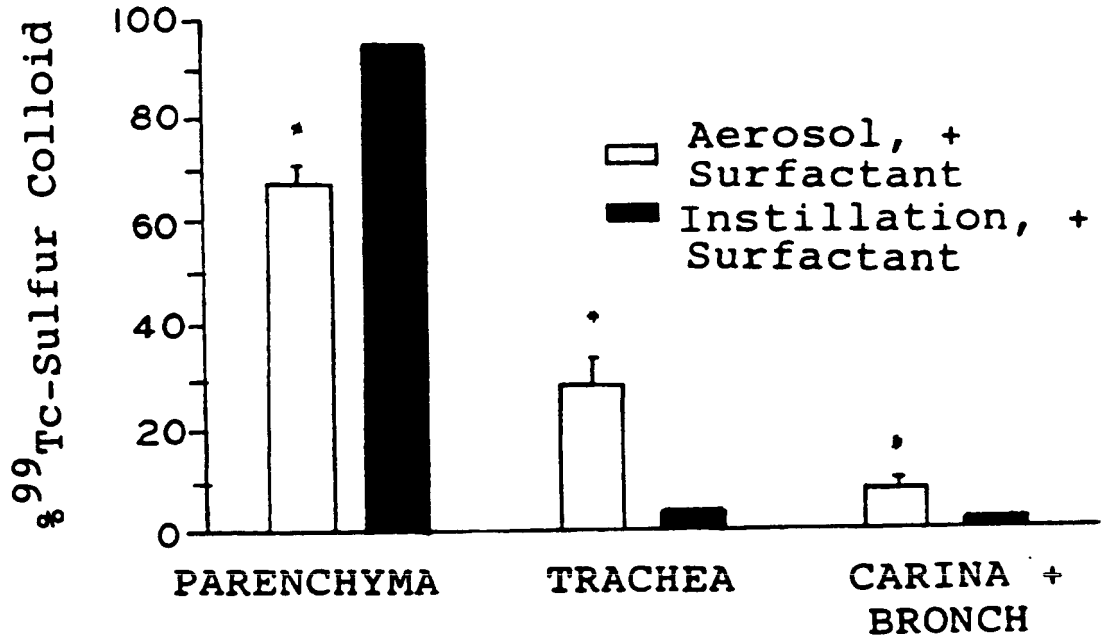


FIG. 17A

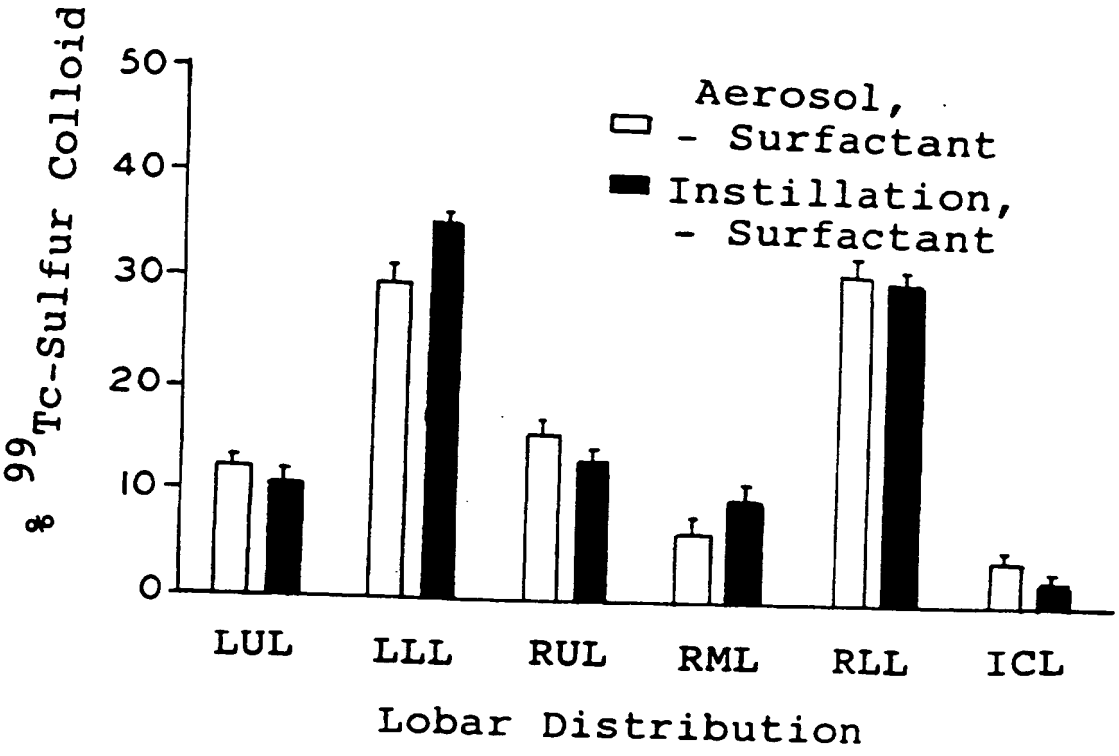


FIG. 17B

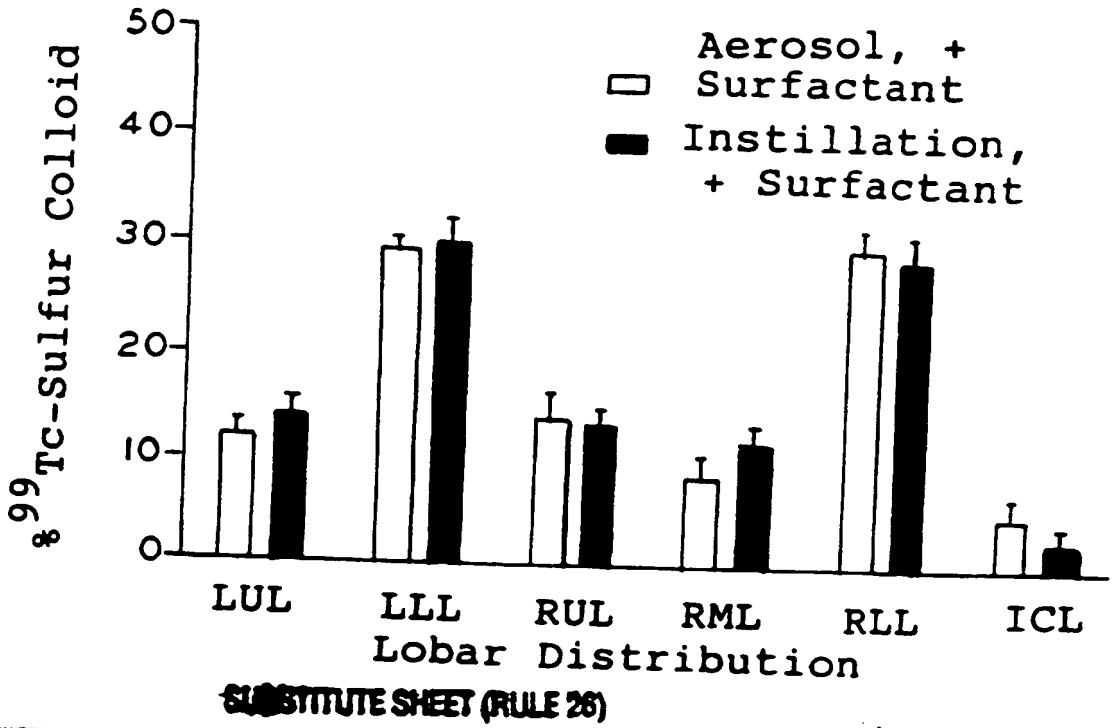


FIG. 18A

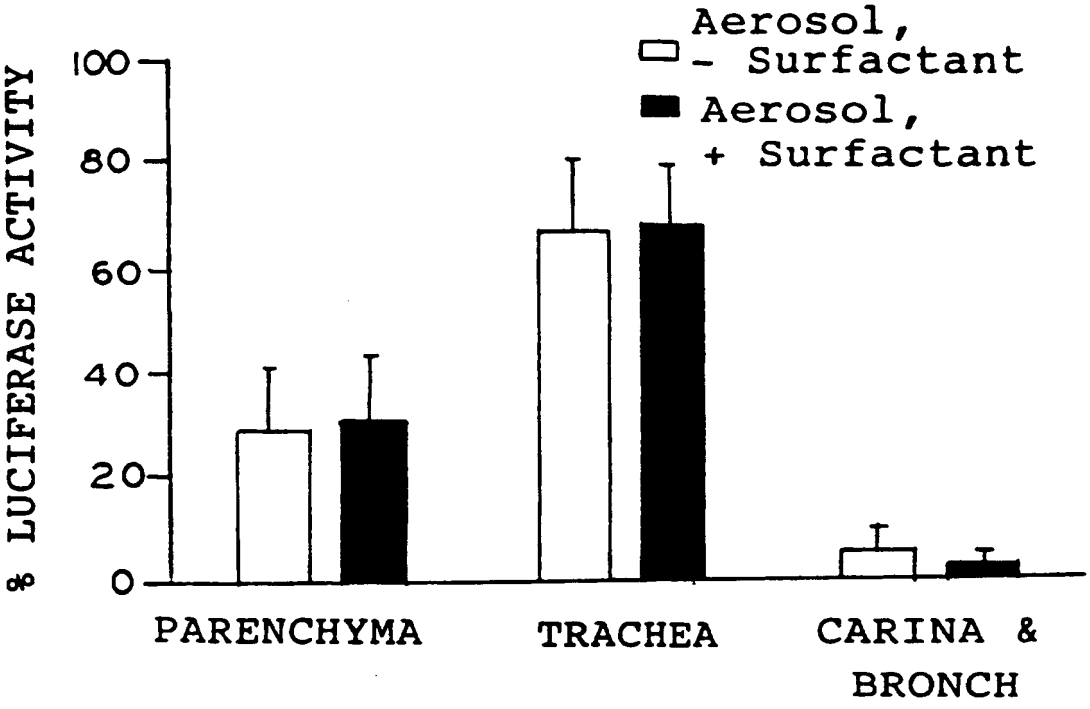
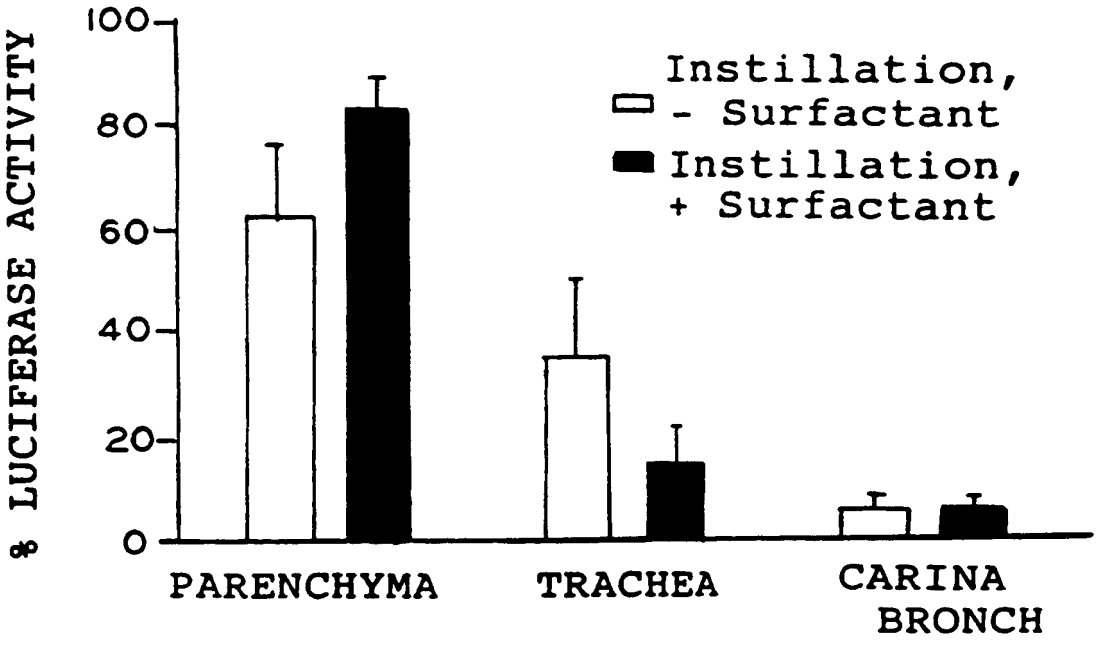


FIG. 18B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04097

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 48/00, 9/127
US CL :514/44; 424/450
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44; 424/450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAATZ et al. Utilization of modified surfactant-associated protein B for delivery of DNA to airway cells in culture. Proceedings of the National Academy of Sciences USA. March 1994, Vol.91, pages 2547-2551, see entire document.	1-4, 8-9, 12, 15-17, 21-22
Y		7, 10-11, 13-14, 23-26
Y	KHARASCH et al. Pulmonary Surfactant as a Vehicle for Intratracheal Delivery of Technetium Sulfur Colloid and Pentamidine in Hamster Lungs. Am. Rev. Respir. Dis. 1991, Vol.144, pages 909-914, see entire document.	11, 25-26
X	ROSS et al. Surfactant Protein A-Polylysine Conjugates for Delivery of DNA to Airway Cells in Culture. Human Gene Therapy. January 1995, Vol.6, pages 31-40, see entire document.	1-4, 8-9, 12, 15-17, 21-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
30 MAY 1996

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04097

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KATKIN et al. A beta-galactosidase adenoviral vector delivered with surfactant. Pediatric Research. 1994, Vol.37, No.4, page 336A, abstract no. 2003, see entire document.	1-4, 9, 11, 15-17, 22, 25
Y	WOLFSON et al. Pulmonary administration of drugs (PAD): A new approach for drug delivery using liquid ventilation. FASEB J. 1990, Vol.4, No.4, page A1105, abstract no. 4868, see entire document.	11, 14, 25
X	KASER et al. Enhanced expression of a human pulmonary surfactant apoprotein A cDNA plasmid in the adult rat lung using cationic liposomes mixed with Exosurf. Pediatric Research. 1994, Vol.37, No.4, page 393A, abstract no. 2340, see entire document.	1-2, 5-6, 8, 11, 13, 15, 18-19, 21, 24-25